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**REMARKS****Provisional Double Patenting Rejection:**

The Examiner rejected claims 1-4, 6-10, 12, 15-17, 24, 28, 32, and 52-55 under the judicially created doctrine of obviousness-type double patenting over U.S. Patent No. 6,498,142. In response, applicants without conceding to the correctness of the Examiner's position but to expedite prosecution of the subject application, will consider filing a terminal disclaimer upon notification of allowable subject matter.

**Rejection under 35 U.S.C. §103**

The Examiner rejected claims 1-4, 6-10, 12 and 52-55 under 35 U.S.C. §103(a) as being obvious over Kuberasampath, Watanabe and Glasscock. The Examiner also rejected claims 1-2, 15 and 16 under 35 U.S.C. §103(a) as being obvious over Kuberasampath, Watanabe and Glasscock, and further in view of Coe, Kees-Folts and Jennerholm. The Examiner also rejected claims 1-2, 17, 24, 28 and 32 under 35 U.S.C. §103(a) as being obvious over Kuberasampath, Watanabe and Glasscock, and further in view of Brenner.

First, Applicants respectfully contend that the Examiner's reasoning on page 5, line 17 through page 6, line 2 is based on false premises and the logic is improperly construed. Specifically, the Examiner recites on page 5, lines 17 -18 that "All mammals, including those with chronic diabetic retinopathy and those without, are at 'risk of chronic renal failure'" (emphasis added). Diabetic retinopathy, which may result in blindness, is a condition marked by retinal vascular microaneurysms and blood hemorrhages in the early nonproliferative phase, and by neovascularization in the later proliferative phase, see Harrison's Principles of Internal Medicine, 15th ed./editors E. Braunwald et al., McGraw-Hill, 2001, p2121. The Examiner has provided no link between diabetic retinopathy, an eye disorder, and chronic renal failure. In addition to objecting to the Examiner's alleged link between diabetic retinopathy and chronic renal failure, applicants contend that the statement by the Examiner that "All mammals ...are at 'risk for chronic kidney failure'" is incorrect. The present application defines the term "subjects at risk of chronic kidney failure" in the bridging paragraph between pages 11-12 as follows: "As used herein, a subject is said to be in, or at risk of, chronic renal failure...if the subject is

reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephrons units” (emphasis added). Therefore, based on the specification, not all mammals are at risk for chronic renal failure, but only those reasonably expected to suffer a progressive loss of renal function associated with progressive loss of functioning nephrons units. The Examiner’s statement of who is at risk for chronic kidney failure is incorrect and inconsistent with the definition provided in the specification. Therefore, the Examiner’s conclusions that the claimed invention is prima facie obvious is in view of the cited references inappropriately based on the Examiner’s incorrect statements.

In addition, applicants note that the Examiner states on page 5, lines 18-22, that since “[a]ll mammals including those with chronic diabetic retinopathy and those without, are ‘at risk of chronic renal failure,’” then “[i]t follows then that treating chronic diabetic nephropathy with OP-1 would improve renal function in a mammal afflicted with chronic diabetic nephropathy, in the absence of evidence to the contrary, because there is no difference in the presently claimed method steps and the method steps taught by the prior art.” Applicants respectfully submit that not only is this statement based on the incorrect premise that all mammals are at risk of chronic kidney failure, but the reasoning is flawed. It is unclear how the alleged lack of difference between the claimed method steps and the teachings in the cited art leads to the conclusion that OP-1 improves renal function, especially when no cited reference teaches that administration of OP-1 improves kidney function. Again, given that the Examiner has based his reasoning on faulty premises and has failed to provide specific support to establish a prima facie case of obviousness, applicants respectfully request that the Examiner withdraw this ground of rejection.

A. Rejection of Claims 1-4, 6-10, 12 and 52-55 under 35 U.S.C. §103(a)

The Examiner rejected claims 1-4, 6-10, 12 and 52-55 under 35 U.S.C. §103(a) as being obvious over Kuberasampath, Watanabe and Glasscock. The Examiner alleges that the three references together provide a teaching, suggestion or motivation to treat chronic diabetic nephropathy with OP-1. Applicants respectfully traverse.

1. Watanabe

As pointed out by the Examiner, Watanabe contains the following statement on the first paragraph of the introduction, page 1, column 1: “Neutrophilic polymorphonuclear leukocytes

(PMN) are important effector cells in glomerular diseases, including IgA nephropathy and diabetic nephropathy (1-3).” The three references cited in Watanabe in support of this statement are Sanders et al., Clin Sci Mol Med 54: 667-672, 1978 (“Sanders”) (Exhibit A), Chen et al. J Clin Lab Anal 6:35-39, 1992. (“Chen”) (Exhibit B), and Nath et al. Diabetes 33:536-589, 1984 (“Nath”) (Exhibit C). However, as it relates to diabetic nephropathy, this statement is unfounded in the cited literature. Sanders describes the involvement of PMN proteinase in glomerulonephritis. Sanders contains no teachings or references to diabetes, and certainly none to diabetic nephropathy. Chen describes the production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by neutrophilic polymorphonuclear leukocytes (PMN) after stimulation and the infiltration of PMN in the glomeruli of patients afflicted with primary IgA nephropathy. Chen lacks any teachings or references to diabetic nephropathy. Nath describes the levels of superoxide anion in PMNs from diabetic and nondiabetic patients. However, the patients in this study do not suffer from diabetic nephropathy. Referring to the patients who participated in the study, the Materials and Methods section of Nath, specifically the last sentence of page 596, states that: “None of the above groups were underweight or malnourished, obese, or had any renal or other complication (or infection) at the time of the study” (emphasis added). In fact, Nath is completely silent with respect to diabetic nephropathy or any other type of renal disorder. Thus, the statement cited by the Examiner in Watanabe is merely an isolated speculation by the authors, lacking factual support.

Contrary to this unfounded statement in Watanabe, the scientific and medical literature at the time of the invention indicated that the pathology of diabetic nephropathy was due to the high blood sugar (hyperglycemia) found in diabetics, and not to an inflammatory response as described in more detail below. The literature taught that diabetic nephropathy was caused by a TGF- $\beta$ -dependent thickening of the extracellular matrix of the kidney. The literature indicated that this thickening was induced by glycation end products formed as a result of the high glucose levels, and not induced by an inflammatory response.

For example, Makino et al., Nephrol Dial Transplant. 1996; Suppl 5:76-80 (Exhibit D, abstract only), provides a mechanistic link between high sugar levels and glycation end products. In the abstract, Makino recites: “Available data indicate that the development of diabetic nephropathy is linked to hyperglycemia. Glucose reacts nonenzymatically with proteins to form a Schiff base and Amadori products. Further incubation of these early products leads to the formation of advanced glycation end-products (AGEs). AGEs seem to play a central role in the



progression of diabetic nephropathy...As the AGEs are localized most notable in nodular lesions, advanced glycations play a role in the progression of diabetic nephropathy through impairment of the assembly of matrix proteins in vivo.”

Iwano et al. *Kidney Int.* 1996; 49(4):1120-6 (Exhibit E, abstract only), recite in the abstract that “Transforming growth factor-beta-1 (TGF-beta 1) is a primary determinant of the mesangial expansion observed in the diabetic nephropathy...These data suggest that the hyperglycemia induces intraglomerular TGF-beta 1 expression in vivo, and that TGF-beta 1 overproduction may be associated with the progression of diabetic nephropathy.” Likewise, Striker et al., *Diabetes & Metabolism* 1996, 22 (Exhibit F), 407-414, recite in the conclusion section on page 413, first column, that “There is considerable evidence that the deleterious effects of hyperglycemia may be mediated by advanced glycosylation end-products.”

Consistent with a hyperglycemic and not an inflammatory pathological basis for diabetic nephropathy, the medical and scientific literature at the time of the invention advocated the control of hypertension and hyperglycemia for treating or preventing diabetic nephropathy rather than the administration of anti-inflammatory agents. Bretzel et al., *J Diabetes Complications*, 1997, 11(2):112-22 (Exhibit G, abstract only), recites in the abstract: “The main risk factors for the frequency, severity, and progression of diabetic nephropathy are the degree of hyperglycemia and associated metabolic disturbances, hypertension, protein overload, cigarette smoking, as well as the duration of diabetes. Interventional strategies for primary, secondary, and tertiary prevention of diabetic nephropathy therefore include meticulous glycemic control, appropriate treatment of associated lipid abnormalities, rigorous control of the blood pressure, reduction in dietary protein intake, in particular animal protein, and of fat intake, and stopping cigarette smoking.” Likewise, Friedman et al., *Chronic Complications in Diabetes*, 25(2), pages 293-324 (Exhibit H) recommended blood pressure normalization, dietary protein restriction and glycemic control to retard the progression of diabetic nephropathy.

Thus, applicants have shown that (i) Watanabe contains an unfounded and unsupported statement from which the Examiner bases his argument; (ii) the literature at the time of the invention taught that diabetic nephropathy was based on a TGF- $\beta$ -dependent thickening of the extracellular matrix due to hyperglycemia; and (iii) the literature at the time of applicant's filing taught away from treating diabetic nephropathy, a non-inflammatory renal condition, with an anti-inflammatory protein, and instead suggested regulating hyperglycemia and hypertension. Accordingly, there was no reasonable expectation of success based on the teachings of Watanabe

and others presented by the Examiner for using OP-1 to treat diabetic nephropathy or chronic kidney failure, and the remaining references do not themselves provided a teaching, suggestion or motivation.

## 2. Glasscock

The Examiner's reliance on Glasscock to support his argument is improper because its teachings are unrelated to diabetic nephropathy.

According to the Examiner, Glasscock teaches that macrophages (monocytes) are present in large numbers in the glomerulus and interstitium in many forms of glomerulonephritis and tubulointerstitial nephritis, and that accordingly, interference with the accumulation of these cells within the kidney may ameliorate the clinical and morphological manifestations of the disease. The two kidney disorders referenced in Glasscock, glomerulonephritis and tubulointerstitial nephritis, are two forms of renal nephritis. The term "nephritis" means "inflammation of the kidney" (Dorland's Illustrated Medical Dictionary, ed D. Andersons, 29th edition, W.B. Saunder's Company. 2000). Glasscock only teaches that macrophages are accumulated in two types of renal nephritis, but not in other forms of kidney disorders which are nephritis-based. Glasscock does not teach or suggest that macrophages are present in large numbers in the glomerulus and interstitium in patients suffering from non-inflammatory kidney disorders and certainly does not teach that macrophages are accumulated in this fashion in patients specifically suffering from chronic diabetic nephropathy.

In his reasoning, the Examiner uses Glasscock as the basis to provide a reasonable expectation for success. The Examiner recites on page 8, lines 11-16 "One of ordinary skill in the art would have a reasonable expectation of success because Glasscock teaches that the monocytes (macrophages) are present in large numbers in the glomerulus and intersitium in many forms of glomerulonephritis and tubulointerstitial nephritis; interference with the accumulation of these cells within the kidney may ameliorate the clinical and morphological manifestation of the disease." However, as described in the preceding paragraph, Glasscock does not provide an expectation of success because Glasscock only teaches that macrophage accumulation occurs in nephritis, and fails to teach or suggest that accumulation is present in non-nephritis kidney disorders, much less in diabetic nephropathy.

Not only does Glasscock fail to provide a suggestion the macrophages are accumulated in large numbers in the glomerulus and interstitium in patients suffering from diabetic nephropathy,

but the medical literature at the time of the invention taught that no such accumulation of macrophages existed. Hooke at al., *Kidney Int.* 1987;31(4):964-72 ("Hooke") (Exhibit I) teaches that there is no statistical significance in the number of intraglomerular or interstitial macrophages (monocytes) in normal subjects vs. those afflicted with diabetic nephropathy (page 967, table 2, and page 968, table 5, respectively). Accordingly, one skilled in the art would have had no reasonable expectation of success of treating diabetic nephropathy with an agent that inhibits macrophage accumulation when no macrophage accumulation exists in subjects suffering from diabetic nephropathy. By teaching a lack of macrophage accumulation in patients from diabetic nephropathy, Hooke negates the Examiner's interpretation of Glassock.

Thus, The Examiner has improperly relied on Watanabe and Glassock to support his argument for prima facie obviousness. The remaining reference, Kuberasampath, does not itself provide a teaching, suggestion or motivation for using OP-1 to treat diabetic nephropathy and therefore the Examiner has failed to make a case of prima facie obviousness. Applicants respectfully request that the Examiner withdraw this ground rejection.

### 3. Kuberasampath, Watanabe and Glassock in Combination

Using Kuberasampath, Watanabe and Glassock, the Examiner sets forth the following case of prima facie obviousness:

- a. Kuberasampath teaches that damage to cells resulting from the effects of inflammatory response has been implicated as the cause of reduced tissue function or loss of tissue function in diseases of the kidney.
- b. Kuberasampath teaches that glomerular nephritis and diabetes are believed to result in large part from unwanted acute inflammatory reaction and fibrosis.
- c. Kuberasampath teaches that OP-1 inhibits the adherence of inflammatory LTB<sub>4</sub> activated PMNs to endothelium and inhibit cellular and humoral inflammatory reactions.
- d. Watanabe teaches that PMNs are important effector cells in glomerular diseases, including diabetic nephropathy.

e. Glassock teaches that large numbers of monocytes (macrophages) are present in large numbers in the glomerulus and interstitium in many forms of glomerulonephritis and tubulointerstitial nephritis, and interference with this accumulation may ameliorate the clinical and morphological manifestations of the disease.

The Examiner argues that since OP-1 reduces macrophage accumulation, then one skilled in the art would have been motivated to use the anti-inflammatory OP-1 protein to treat chronic renal failure in a subject afflicted with diabetic nephropathy, allegedly caused by an accumulation of inflammatory macrophages in the kidney. However, applicants have shown that Watanabe and Glassock do not teach that macrophages are accumulated in diabetic nephropathy, as these references relate to nephritis and not to diabetic nephropathy. Applicants have further shown that Hooke teaches, through direct examinations of patients, that there is no accumulation of monocytes (macrophages) in the kidneys of patients suffering from diabetic nephropathy. Thus, one skilled in the art would have had no motivation to combine the teachings of Kuberasampath (*i.e.* that OP-1 inhibits macrophage adherence) to treat a disease, such as diabetic nephropathy, which is not caused by macrophage accumulation. The Examiner incorrectly infers from Kuberasampath that if some forms of diabetes are caused by excessive inflammatory responses, then it follows that any disorder found in diabetic patients must also be caused by an excessive inflammatory response. Thus, the Examiner infers from Kuberasampath that diabetic nephropathy must be caused by excessive inflammation. The Examiner then improperly relies on the teachings of Watanabe and Glassock (that reducing inflammation in renal diseases caused by inflammation will improve renal function) to argue that reducing inflammation in patients with diabetic nephropathy will improve renal function in these patients by reducing inflammation.

Accordingly, the Examiner has overextended the combined teachings of Kuberasampath, Watanabe and Glassock and failed to make a case of *prima facie* obviousness. Applicants respectfully request that the Examiner withdraw this ground of rejection.

#### 4. Lack of Reasonable Expectation of Success

Not only have applicants shown that Kuberasampath, Watanabe and Glassock fail to provide a teaching, suggestion, motivation, or reasonable expectation of success for using the anti-inflammatory agent OP-1 to treat chronic diabetic nephropathy, but the medical and scientific literature at the time the present application was filed taught that the administration of

widely used anti-inflammatory agents, including salicylates (aspirin), propionic acids (ibuprofen), indolacetic acids (indomethacin) and anthranilic acids, could result in kidney failure, particularly in patients suffering from preexisting impairments in kidney function. For example, the review article Whelton et al. (1991) Clin Pharmacol. 31(7):588-98 (Exhibit J), teaches that "approximately 1-5% of people who are exposed to a nonsteroidal anti-inflammatory drug (NSAID) will manifest one of a variety of renal function abnormalities ... Renal abnormalities include fluid and electrolyte disturbances, acute deterioration of renal function, nephritic syndrome with interstitial nephritis, and papillary necrosis" (Page 588, columns 1-2). Furthermore, Whelton et al. teaches that "from the clinical point of view, the most worrisome renal side effect of NSAIDs is hemodynamically mediated acute renal failure, which occurs in individuals with pre-existing reduced renal blood perfusion" (Page 588, column 2).

The review article Bennet et al. (1996) Am J Kidney Dis 1996 28 (1 Suppl 1):S56-62 (Exhibit K) provides recommendations based on a critical literature survey. Bennet concludes that while the use of NSAIDs in the general population is safe and effective when used in therapeutic dosages for a limited period of time, "patients with pre-existing risk factors are susceptible to potentially life-threatening toxicities, including acute renal failure (ARF) and serious fluid and electrolyte disorders" (page S-61, column 1). Similarly, another review article by Murray et al., (1997) Prog Drug Res. 49:155-71 (Exhibit L, abstract only), warns of the risk of administering anti-inflammatory agents to patients with abnormal kidney function in the abstract: "Among persons with normal renal function, who have no other risk factors (dehydration) for an acute hemodynamic effect, there is no risk. However, NSAID administration to susceptible persons may cause decrements in renal plasma flow and glomerular filtration rate within hours" (emphasis added).

Therefore, because the teachings at the time of the invention warned of the possible renal disorders that could be caused by a broad class of anti-inflammatory agents, a skilled artisan at the time of the invention was made would not have had a reasonable expectation of success of using an anti-inflammatory agent such as OP-1 for the treatment of chronic kidney failure or for diabetic nephropathy. In fact, a skilled artisan would have expected a worsening of the kidney failure in patients at risk of or suffering from diabetic nephropathy.

B. Rejection of Claims 1-2, 15 and 16 under 35 U.S.C. §103(a)

The Examiner has rejected claims 1-2, 15 and 16 under 35 U.S.C. §103(a) as being obvious over Kuberasampath, Watanabe and Glasscock, and further in view of Coe, Kees-Folts and Jennerholm. The Examiner argues that claims 1 and 2 are obvious based on Kuberasampath, Watanabe and Glasscock. The Examiner then uses references Coe, Kees-Folts and Jennerholm to support his case of obviousness for claims 15 and 16, which are dependent on claim 1. The basis for the Examiner's reasoning is provided in the November 18, 2002 Office Action.

Applicants have shown in section A that Kuberasampath, Watanabe and Glasscock do not form the basis for a prima facie case of obviousness with respect to claims 1, 2. Furthermore, Coe, Kees-Folts and Jennerholm do not provide a teaching, suggestion, motivation, or expectation of success on their own, or when combined with Kuberasampath, Watanabe and Glasscock, to treat diabetic nephropathy with OP-1. Accordingly, since claims 1 and 2 are not obvious over the prior art, and claims 15 and 16 directly or indirectly depend on claim 1, claims 15 and 16 are not obvious.

The teachings of Coe, Kees-Folts and Jennerholm fail to provide the missing motivation, teaching or suggestion to treat chronic diabetic nephropathy with OP-1, as recited in claims 1 and 2, when combined with Kuberasampath, Watanabe and Glasscock. The Examiner states on page 7, first paragraph of the November 18, 2002 Office Action that Kees-Folts teaches that "interstitial macrophage infiltration has been identified in diabetic nephropathy (page 366, paragraph bridging columns 1-2)." The exact sentence referred to by the Examiner recites as follows: "Even more striking is the number of disorders in which interstitial macrophage infiltration has been identified; the list includes...diabetic nephropathy... among others [12]." Applicants point out that the sole reference in support of this statement, reference 12, corresponds to the Hooke reference. As described in section A above, Hooke teaches that there is no statistical significance in the number of intraglomerular or interstitial macrophages (monocytes) in normal subjects vs. those afflicted with diabetic nephropathy (page 967, table 2, and page 968, table 5, respectively). Accordingly, Kees-Folts fails to provide evidence of an accumulation of interstitial macrophages in patients with diabetic nephropathy, and in fact makes reference to a study that directly contradicts this allegation.

Because a case of prima facie obviousness has not been made by the Examiner with respect to claims 1 and 2, or with respect to claims 15 and 16, applicants respectfully request that the Examiner withdraw this ground of rejection.

C. Rejection of Claims 1-2, 1-2, 17, 24, 28 and 32 under 35 U.S.C. §103(a)

The Examiner has rejected claims 1-2, 17, 24, 28 and 32 under 35 U.S.C. §103(a) as being obvious over Kuberasampath, Watanabe and Glasscock, and further in view of Brenner. The Examiner as set forth in the Office Action dated November 18, 2002 argues that claims 1 and 2 are obvious based on Kuberasampath, Watanabe and Glasscock. The Examiner then uses the Brenner reference to support his case of obviousness for claims 17, 24, 28 and 32, which are dependent on claim 1.

Applicants have shown in section A of this response that Kuberasampath, Watanabe and Glasscock do not form the basis for a prima facie case of obviousness with respect to claims 1, 2, and the Brenner reference does not provide the missing elements. Accordingly, since claim 1 is not obvious over the cited references, and claims 17, 24, 28 and 32 directly or indirectly depend on claim 1, claims 17, 24, 28 and 32 are not obvious. Accordingly, applicants respectfully request that the Examiner withdraw this ground of rejection.

D. Conclusions

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

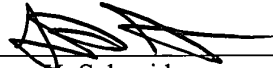
Application No.: 08/851628

Docket No.: JJJ-P01-515

No fee other than the \$110.00 one-month extension of time fee is deemed necessary in connection with the filing of this application. Authorization is hereby given to charge the \$110 fee and any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 18-1945, under Order No. JJJ-P01-515 from which the undersigned is authorized to draw.

Dated: December 12, 2003

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## Lysosomal enzymes in human urine: evidence for polymorphonuclear leucocyte proteinase involvement in the pathogenesis of human glomerulonephritis

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### Summary

1. Lysosomal proteinase activity was assayed in human cadaver kidney, urine, granules from polymorphonuclear leucocytes of normal persons, and urine samples from 154 patients with renal disease.

2. Granules from polymorphonuclear leucocytes showed proteinase activity at acid and neutral pH, whereas cadaver kidney showed proteinase activity at acid pH only.

3. The urine from 13 patients with glomerulonephritis showed proteinase activity at both acid and neutral pH as well as increased amounts of antigenic glomerular basement membrane fragments. The properties of the urinary proteinases suggested that they had originated in polymorphonuclear leucocytes.

4. Only the urine samples containing these proteinases were capable of degrading isolated human glomerular basement membrane *in vitro*.

5. Clinical recovery, where it occurred, was accompanied by the disappearance of urinary proteinase activity, and reduction in glomerular basement membrane antigen excretion.

**Key words:** basement membrane, glomerulonephritis, lysosomes, polymorphonuclear leucocytes, proteinases.

**Abbreviation:** GBM, glomerular basement membrane.

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### Introduction

Animals with nephrotoxic nephritis (Hawkins & Cochrane, 1968; Cochrane & Aikin, 1966) excrete acid proteinases of lysosomal origin in their urine during the first (heterologous) phase of the disease process, which suggests that the glomerular injury may be due to lysosomal enzymes released from polymorphonuclear leucocytes. However, there is no direct evidence to confirm that this mechanism occurs in human glomerulonephritis. We have therefore studied the possible role of polymorphonuclear leucocyte lysosomal enzymes in human renal disease by examining the characteristics of proteinase excretion in normal and pathological urine, together with similar assays for plasma, kidney and leucocyte enzymes. We have also studied isolated human glomerular basement membrane *in vitro* to identify the potential pathogenicity of the urinary proteinases.

### Materials and methods

#### Patients and urine samples

Of the 154 patients with renal disease, 97 had glomerulonephritis. Diagnosis was based upon clinical and/or histopathological criteria. Renal biopsy was only undertaken when there was a specific clinical indication. Standard techniques of fixation and staining for light and electron microscopy and immunofluorescence were used. In 18 of those with glomerulonephritis, diagnosis was based upon clinical and biochemical findings alone. Of the remaining 79 with glomerulonephritis, 31 had

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diffuse proliferative disease, 25 had membranous glomerulonephritis, five minimal change, 16 mesangiocapillary and two mesangiopathic (IgA) nephropathy. The other 57 patients without glomerulonephritis had a variety of diagnoses including polycystic kidneys, pyelonephritis and renal transplant rejection.

Both 24 h and random samples, taken throughout the day from the patients and from 21 healthy control subjects, were concentrated up to 15-fold by using an Amicon thin-channel separator with UM10 membranes. The concentrated samples were stored at  $-20^{\circ}\text{C}$  until assayed. More than one sample was taken from both patients and control subjects and assayed.

#### Polymorphonuclear leucocyte

Intact granules from the polymorphonuclear leucocytes of healthy laboratory personnel were prepared as described by Davies, Barrett, Travis, Sanders & Coles (1978).

#### Analytical methods

**Protein.** Total protein concentrations were determined by a Folin-Lowry technique on a Technicon Auto-analyzer with crystalline bovine serum albumin (factor V Armour) used as reference standard.

**Acid proteinase.** This was measured by the method of Anson (1939) as modified by Barrett (1967). The effect of pH on acid proteinase activity was determined over the range pH 2.1–6.0 (Barrett, 1967). The liberated peptides, soluble in trichloroacetic acid, were determined by their Folin-Lowry reaction.

**Neutral proteinase.** Total neutral proteinase activity was assayed with azocasein as substrate (Starkey & Barrett, 1976). Elastase (EC 3.4.21.11) activity was assayed with elastin from bovine nuchal ligament (Sigma) as substrate over the range pH 6–10 (Ohlsson & Olsson, 1974) and with *N*-benzyloxycarbonyl-L-alanine 2-naphthyl ester (Z-Ala-2-O-Nap) (kindly donated by Dr C. G. Knight, Strangeways Research Laboratories, Cambridge). Cathepsin G (EC 3.4.21.20) activity was assayed against *N*-benzoyl-DL-phenylalanine 2-naphthyl ester (BZ-DL-Phe-2-O-Nap) (Sigma) as substrate (Starkey & Barrett, 1976). The effects of enzyme activators and inhibitors were investigated by the addition of the appropriate compound in the standard assay mixture.

Enzyme activities are defined as units of product liberated per unit time.

#### Glomerular basement membrane

**Preparation of antigen.** Anti-(human glomerular basement membrane) was produced in male New Zealand White rabbits by intramuscular injection of human glomerular basement membrane (GBM) isolated from cadaver kidneys (Krakower & Greenspon, 1951). Serum was stored at  $-20^{\circ}\text{C}$ . GBM antigen in urine was assayed by a double-diffusion technique in agarose [1.0 g/100 ml (Calbiochem)] in phosphate-buffered saline, pH 7.2, over 72 h. Results were expressed as the minimum concentration of urine which produced a precipitin line on completion of the incubation period. The antigenic fragments present in the urine of two patients were further investigated by separation on a Sephadex G-200 column.

**Degradation.** Freeze-dried human GBM was incubated with urine (2 mg/ml) as enzyme source. The release of soluble hydroxyproline was used as a measure of the extent of degradation as previously described by Davies *et al.* (1978).

#### Results

##### Urinary enzyme excretion

**Normal subjects.** All normal subjects excreted various amounts of an acid proteinase (mean =  $50.26 \pm 42.63$  units/ml of urine,  $n = 43$ ); the pH profile is shown in Fig. 1. The enzyme had a maximum activity at pH 2.1 and this, together with the finding of complete inhibition of activity by pre-incubation in alkaline conditions, suggests that the enzyme was pepsin (EC 3.4.23.1). The activity in healthy persons at pH 3.4, the optimum for cathepsin D (EC 3.4.23.5), a lysosomal proteinase, was always less than 50% of that at pH 2.1. Therefore, for screening purposes, urine proteinase activity was measured at pH 2.1 and 3.4. Those samples in which activity at pH 3.4 was equal to or greater than 50% of activity at pH 2.1 were further investigated with a full profile of activity over the range pH 2.1–6.0.

**Patients.** Total acid (pH 2.1) proteinase was significantly reduced in renal disease. Mean activity was  $2.56 \pm 2.76$  units/ml of urine,  $n = 252$  ( $n$  = total number of assays performed) when compared with normal subjects, and by Student's *t*-test this was highly significant ( $P < 0.001$ ).

The pattern of proteinase excretion was normal in all but 13 patients, who all showed additional activity at pH 3.4 equal to or greater than 50% of activity at pH 2.1, with a bimodal distribution of

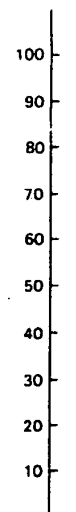


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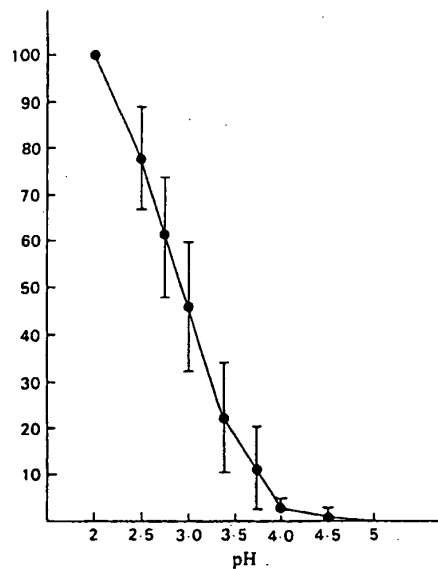


FIG. 1. Proteinase activity of normal urine at different pH values. Results have been expressed as % of activity at pH 2.1. Each point is the mean value and SD for 21 subjects.

acid proteinase activity with varying pH. Activity at pH 3.4 =  $3.2 \pm 3.22$  units/ml,  $n = 54$ . In addition, all 13 excreted a third proteinase ( $3.03 \pm 3.68$  units/ml,  $n = 55$ ) with optimum activity at neutral pH (Fig. 2) when assayed against elastin or azocasein. Neutral proteinase activity could not be demonstrated in urine samples from any other patients, despite the presence of numerous leucocytes in some urine samples.

#### Excretion of GBM antigen

GBM antigen was detectable in normal urine only after concentrations to at least 75-fold. How-

ever, all the previously mentioned 13 patients with glomerulonephritis had demonstrable antigenic fragments when the urine was concentrated only 15-fold, and serial dilution revealed similar fragments, in some patients when samples were concentrated only 7.5- to 1.5-fold. Antigen titres in all other patients were similar to those in normal subjects. On immunodiffusion, there appeared to be at least three distinct antigens present in both normal urine and pathological urine. Subsequent examination of two of these samples by gel chromatography indicated that the molecular weights of these three antigens were 80 000, 150 000 and in excess of 200 000.

#### Clinical results

Of the 13 patients with abnormal patterns of enzyme and GBM excretion, one had a steroid-responsive nephrotic syndrome. The remaining 12 had acute oliguric, or rapidly progressive glomerulonephritis. In all 12 renal biopsy revealed a diffuse proliferative glomerulonephritis with many polymorphonuclear leucocytes present in the glomeruli. Whereas in normal kidneys only the occasional polymorphonuclear leucocyte is seen in any glomerular tuft, these 12 patients had at least three and often as many as 15 polymorphonuclear leucocytes per glomerulus. In all other biopsy samples from patients the counts of polymorphonuclear leucocytes per glomerulus were less than three with an average of 1.5. The biopsies from the 12 patients with proliferative glomerulonephritis showed extracapillary crescents in six; immunofluorescence studies in eight of them showed linear IgG deposits in five, and linear  $\beta$ 1C

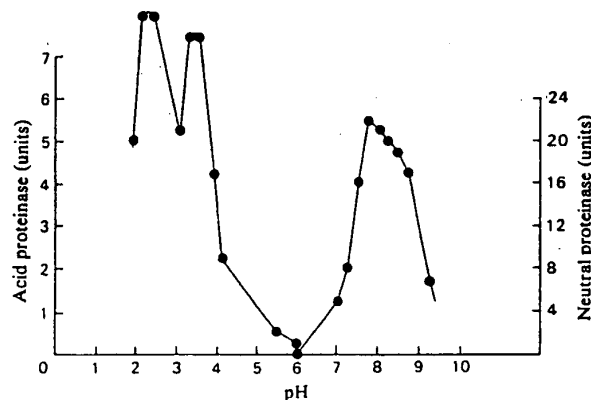


FIG. 2. Profile of urinary proteinase activity from pH 1 to pH 10 in patient O.S., who had rapidly progressive glomerulonephritis with cellular proliferation and glomerular crescents.

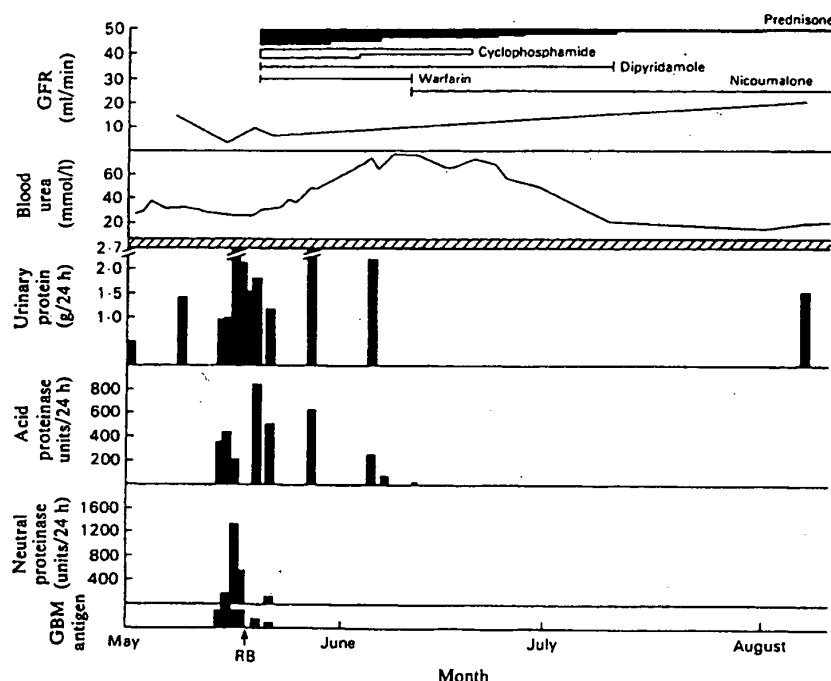


FIG. 3. Clinical course of patient D.L. (age 44 years) with rapidly progressive glomerulonephritis. All assays were performed on 24-h urine collections. Each point marks one complete assay; collections were not possible during the period July–August. Enzyme and GBM antigen disappeared from urine together during the month of June and did not reappear despite the persistence of severe proteinuria. GFR, Glomerular filtration rate; RB, renal biopsy.

in four (two had both linear IgG and  $\beta$ 1C) and granular deposition of IgG in one.

Subsequent, spontaneous improvement (as judged by determinations of blood urea and glomerular filtration rate) was seen in three patients, and was accompanied by the disappearance of acid (pH 3.4) proteinase and neutral proteinase from the urine, together with a fall in GBM antigen titre. These changes are shown for one patient in Fig. 3.

#### Properties of urinary proteinases

Incubation of the urine samples in the presence of known activators and inhibitors of proteinase activity indicated that the acid (pH 3.4) proteinase was a carboxyl proteinase, since it was unaffected by metal ions, cysteine, mercaptoethanol, iodoacetamide, soyabean trypsin inhibitor, Trasylol and 4-chloromercuribenzoate. Its molecular weight was approximately 50 000, as determined by column chromatography. The neutral proteinase activity contained two enzymes, elastase and cathepsin G, as shown by activity against specific substrates. The elastase was inhibited by Trasylol, serum and soyabean trypsin

inhibitor, and had a molecular weight of 28 000. These results, taken together, suggest that the proteinase activity is derived from lysosomal cathepsin D, elastase and cathepsin G.

#### GBM degradation

The incubation of urinary neutral proteinase (0.250 unit/ml) with freeze-dried human GBM resulted in the solubilization of up to 60% of the total available hydroxyproline. The release of hydroxyproline was linear over the incubation period of 72 h. All other urine samples failed to release hydroxyproline from GBM, as did urinary acid (pH 3.4) proteinase.

#### Tissue enzyme studies

Normal human kidney contained an acid proteinase (pH 3.4) ( $0.53 \pm 0.046$  unit/g wet weight,  $n = 9$ ) but no neutral proteinase. Polymorphonuclear leucocyte granules were shown to have both an acid (pH 3.4) proteinase ( $4.16 \pm 0.49$  units/ $10^9$  polymorphonuclear leucocytes,  $n = 6$ ) and neutral proteinase activity ( $0.386 \pm 0.053$  unit/ $10^9$  polymorphonuclear leucocytes,  $n = 6$ ).

#### Discussion

There was reduction in disease. This with either filtration rate of the urine of proliferative urine of nor with other these enzyme glomerular understanding.

The enzyme serum, the blood. Serum activity, and detectable leucocytes (Janoff & Booyse, 1976) as we most likely.

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## Discussion

There was a highly significant ( $P < 0.001$ ) reduction in uropepsinogen excretion in renal disease. This did not seem to correlate in any way with either the type of disease or with glomerular filtration rate. Neutral proteinases were detected in the urine of 13 of 31 patients with severe, diffuse, proliferative glomerulonephritis, but not in the urine of normal subjects or in the urine of patients with other forms of renal disease. The origin of these enzymes, and their ability to initiate glomerular damage, may be important to the understanding of human glomerulonephritis.

The enzyme activity could enter urine from serum, the urinary tract or the cellular elements of blood. Serum contains no detectable free proteinase activity, and kidney contains cathepsin D but no detectable neutral proteinase. Polymorphonuclear leucocytes and platelets contain neutral proteinases (Janoff & Scherer, 1968; Legrand, Caen, Booyse, Rafelson, Robert & Robert, 1973; Ohlsson & Olsson, 1974; Starkey & Barrett, 1976) as well as cathepsin D, and are therefore the most likely source of this urine enzyme activity.

With the exception of the one nephrotic patient mentioned, only those patients with more than three polymorphonuclear leucocytes per glomerulus had detectable urinary neutral proteinase activity. In all other biopsies taken, glomerular counts of these leucocytes were less than three per glomerulus, and although some patients with rejecting transplants, or severe pyelonephritis, were studied, none of these had elastase or cathepsin D in their urine. This would suggest that the enzymes are released from glomerular polymorphonuclear leucocytes and that enzyme activity is not merely the result of contamination of urine by leucocytes along the urinary tract, as may occur in severe infection. Our failure to detect activity in the urinary deposit may arise from the death of such cells with subsequent neutralization of proteinase activity by cytoplasmic inhibitors. The presence of proteinases and GBM fragments in the urine of the nephrotic patient remains unexplained.

Polymorphonuclear leucocytes contribute to tissue damage in many disease states. Cochrane & Aikin (1966) showed that these leucocytes were necessary for tissue injury in experimental cutaneous Arthus reactions. Hawkins & Cochrane (1968) delayed the onset of proteinuria in experimental nephrotoxic nephritis by preliminary depletion of the leucocytes, an observation that was con-

firmed by Naish, Thomson, Simpson & Peters (1975). These effects of polymorphonuclear leucocytes were attributed to acid proteinases. However, later studies have shown that these leucocytes contain at least three neutral proteinases capable of degrading vascular and glomerular basement membranes (Malemud & Janoff, 1976; Oransky, Ignarro & Perper, 1973). Davies *et al.* (1978) have shown that cathepsin G and elastase damage GBM *in vitro*, whereas cathepsin D had no effect. It therefore seems unlikely that cathepsin D is directly implicated in the pathogenic mechanism, despite previous suggestions to the contrary (Cochrane & Aikin, 1966; Hawkins & Cochrane, 1968; Henson, 1972; Karan, Saatci & Bakkaloglu, 1976).

The studies by Hawkins & Cochrane (1968), Weissman, Zurier & Spieler (1971) and Henson (1972) suggest that the simplest explanation for the damage to the GBM in immunologically induced glomerulonephritis is the selective release of polymorphonuclear leucocyte lysosomal enzymes directly on to the membrane. Our results support this idea, since neutral proteinase was found only in the urine of patients with severe glomerulonephritis, and was accompanied by GBM damage, as shown by the excretion of high concentrations of antigenic fragments. When the patients recovered enzyme and antigen disappeared from the urine. In addition, only urine containing neutral proteinase was capable of causing damage to GBM *in vitro*.

We suggest therefore that polymorphonuclear leucocyte lysosomal neutral proteinases have a significant role in the pathogenic process of rapidly progressive or acute oliguric glomerulonephritis, whether the initial injury is due to the deposition of immune complexes or anti-GBM antibody.

## Acknowledgments

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## Summary

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## Oxidative Metabolism of Polymorphonuclear Leukocytes (PMN) in Patients With IgA Nephropathy

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The production of hydrogen peroxide ( $H_2O_2$ ) by neutrophilic polymorphonuclear leukocytes (PMN) after stimulation and the infiltration of PMN in glomeruli were determined in 20 patients with primary IgA nephropathy. The  $H_2O_2$  production of PMN after the stimulation was measured with a spectrophotometer using horseradish peroxidase as substrate. The results were as follows: 1) when PMN were pretreated with cytochalasin B,  $H_2O_2$  production after stimulation with heat-aggregated IgG (IgG) or serum-treated zymo-

san (STZ) was significantly higher in patients with IgA nephropathy than in controls, and 2) there was an increased amount of PMN localized in glomeruli in patients with IgA nephropathy using immunofluorescence of monoclonal anti-PMN antibody.

It appeared that the increased renal infiltration of PMN which have a high potential for production of reactive oxygen species might induce the glomerular injuries in patients with IgA nephropathy.

**Key words:** reactive oxygen metabolite, polymorphonuclear leukocyte, IgA nephropathy

### INTRODUCTION

Neutrophilic polymorphonuclear leukocytes (PMN) are important effector cells in glomerulonephritis (1-3). PMN-mediated glomerular injuries have documented the critical role of reactive oxygen species—which include superoxide, hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical, and the myeloperoxidase- $H_2O_2$ -halide system—in mediating tissue injuries (2,3).

Human PMN possess various receptors which may, on specific stimulation, release reactive oxygen species and induce tissue injuries. Accumulated evidence has suggested that  $H_2O_2$  may be the most important mediator among the reactive oxygen species released by PMN which damage the kidneys (4,5). Controversial results have been obtained from studies of reactive oxygen species production from PMN in uremic patients (6-9). IgA nephropathy is recognized worldwide as one of the most common glomerulonephritides. IgA nephropathy is characterized by predominant deposition of IgA and C3 in the glomerular mesangial areas based on immunofluorescence and is considered to be an immune-complex mediated glomerulonephritis (10). However, to our knowledge, there have been no studies of the oxidative metabolism of PMN in patients with IgA nephropathy.

The present study was undertaken to determine the roles of reactive oxygen species produced by PMN and the infiltration of PMN in glomeruli in patients with IgA nephropathy.

### MATERIALS AND METHODS

#### Patients

Twenty patients (14 males and 6 females, 27-47 years old) with primary IgA nephropathy (Berger disease) were examined. Patients with predominant mesangial IgA deposits were diagnosed as having IgA nephropathy after exclusion of patients with SLE, Henoch-Schoenlein purpura (HSP) nephritis, cirrhosis, or other systemic diseases. The histological severity in our studies was classified as shown in Table 1 (11). Grade I included four, grade II five, grade III nine, and grade IV two patients with IgA nephropathy in this study. None of the patients was receiving steroids, allopurinol, non-steroidal anti-inflammatory drugs (NSAID), or any immunosuppressive drugs. Twenty healthy adults (13 males and 7 females, 28-36 years old) served as controls. Renal specimens were obtained from 17 patients with IgA nephropathy and 5 normal renal tissues (autopsy cases).

#### Separation of PMN

Leukocyte suspension was prepared by Ficoll-Conray gradients (12). Briefly, heparinized (20 units/ml) venous blood

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**TABLE 1. Histopathological Gratings of IgA Nephropathy**

Grade I ("Minimal"):	Minimal glomerular lesions such as focal segmental thickening of mesangial area
Grade II ("Slight"):	Diffuse mesangial thickening with an increase in the homogeneous PAS-positive mesangial matrix with mild and segmental hypercellularity
Grade III ("Moderate"):	Diffuse mesangial thickening and mesangial cell proliferation with segmental thickening of glomerular capillary walls in certain glomeruli by the extension of the mesangial matrix
Grade IV ("Advanced"):	Capsular adhesion, fibrocellular crescents, glomerular hyalinosis, sclerosis, and severe interstitial changes in addition to grade III

was left at room temperature for 30 minutes after adding normal saline and 5% Dextran-0.9% NaCl solution. The PMN layer was then aspirated into a 10% Ficoll-60% Conray solution and centrifuged. The pellet was washed with phosphate buffered saline (PBS) and then underwent hypotonic lysis of erythrocytes. The PMN were resuspended in PBS and used for the study. This method achieved a purity of PMN as high as  $95 \pm 3\%$  ( $n = 40$ ) without significant contamination of erythrocytes and platelets. Some cells were preincubated with cytochalasin B (5.0  $\mu\text{g/ml}$ ) in 0.1% dimethyl sulfoxide (DMSO) at 37°C for 10 minutes before addition of an appropriate component and stimulant. The final concentration of DMSO was less than 0.1%, which did not interfere with PMN function. Cell viability was tested using the trypan blue exclusion test, which revealed  $97 \pm 3\%$  viable cells.

#### Preparation of Stimulants

Four different stimulants—namely PMA, FMLP, heat-aggregated IgG, and STZ with or without pretreatment of cytochalasin B—were used in this study. Among the stimulants used, PMA was a nonspecific membrane stimulant, FMLP activated the chemoattractant receptor, IgG stimulated the Fc receptors, and STZ reacted with both complement receptors (CR) and Fc receptors because it was opsonized with serum immunoglobulins and C3 fragments. Cytochalasin B, a metabolic product of *Helminthosporium dematioides*, further enhanced the  $\text{H}_2\text{O}_2$  recovery by inhibiting phagocytotic vacuole formation. All the chemicals were purchased from Sigma Chemical (St. Louis, MO, USA) unless mentioned otherwise. PMA was dissolved in DMSO at a concentration of 33.3  $\mu\text{g/ml}$  and stored at  $-80^\circ\text{C}$ . FMLP was dissolved in DMSO at a concentration of  $10^{-4}$  M and stored at  $-20^\circ\text{C}$ . Human IgG was aggregated by heating at  $63^\circ\text{C}$  for 30 minutes, resuspended in PBS buffer at a concentration of 3.0 mg/ml, and stored at  $-80^\circ\text{C}$ . Zymosan was boiled and washed twice with 0.85% NaCl, and then incubated with fresh normal serum at a concentration of 10 mg/ml for 30 minutes at  $37^\circ\text{C}$ . After centrifugation and washing twice, this preparation of serum-treated zymosan (STZ) was suspended in buffer at a concentration of 5 mg/ml. After obtaining a standard dose-response curve, the optimal final concentrations used for stim-

ulation were 80 ng/ml for PMA,  $10^{-6}$  M for FMLP, 600  $\mu\text{g/ml}$  for IgG, and 500  $\mu\text{g/ml}$  for STZ.

#### Det rmination f $\text{H}_2\text{O}_2$ Production

The rate of  $\text{H}_2\text{O}_2$  production was assayed using horseradish peroxidase (HRP) as substrate and carried out in a Hitachi 557 double wavelength, double beam spectrophotometer (13). PBS containing 1 mM  $\text{Ca}^{2+}$  and 2.5  $\mu\text{M}$  HRP was used as the incubation medium. The cuvettes were maintained at  $37^\circ\text{C}$  in a stirring system. An aliquot of PMN suspension with a final concentration of  $10^6$  cells/ml was added to the reaction medium after equilibration at  $37^\circ\text{C}$ . The rate of  $\text{H}_2\text{O}_2$  release into the medium was measured as the rate of formation of HRP- $\text{H}_2\text{O}_2$  compound II by recording the absorption increase at 417–401 nm (molar absorption coefficient =  $50 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ ).

#### Staining for PMN

Renal PMN were reacted with mouse monoclonal anti-human PMN antibody (Sigma Chemical, St. Louis, MO, USA, lot no. 67F-48161; 1:10 dilution) and then stained with FITC-labeled goat anti-mouse Ig antiserum (Cappel Laboratories, Cochranville, PA, USA; 1:20 dilution). FITC-labeled goat anti-mouse Ig antiserum was absorbed with fresh human plasma. The antibody and antiserum were deaggregated at 105,000g for 30 min. The titration of the antibody and antiserum was performed using renal specimens prior to use. Renal PMN were examined with a Zeiss Ortholux microscope (model 9902, Carl Zeiss, Inc., New York, NY, USA).

#### Statistics

Statistical analysis was undertaken by the unpaired *t*-test to compare  $\text{H}_2\text{O}_2$  production in the patients and the controls. The relations between  $\text{H}_2\text{O}_2$  production and histopathological severity or laboratory data (BUN, s-creatinine, CCr, PSP 15 min) were examined by linear regression analysis.

#### RESULTS

##### $\text{H}_2\text{O}_2$ Production by PMA-, FMLP-, IgG-, or STZ- Stimulated PMN

The  $\text{H}_2\text{O}_2$  production of PMN after PMA-, IgG-, or STZ-stimulation in patients with IgA nephropathy was not significantly different from that in normal controls. The  $\text{H}_2\text{O}_2$  production of PMN after FMLP-stimulation in patients with IgA nephropathy was significantly lower than that in normal controls ( $P < 0.05$ ). In preincubation of PMN with cytochalasin B, the  $\text{H}_2\text{O}_2$  production after IgG- or STZ-stimulation in patients with IgA nephropathy was significantly higher than that in normal controls ( $P < 0.05$ ). In preincubation of PMN with cytochalasin B, the  $\text{H}_2\text{O}_2$  production after PMA- or FMLP-stimulation in patients with IgA nephropathy was



TABLE 2. H<sub>2</sub>O<sub>2</sub> Production by PMA-, FMLP-, and STZ-Stimulated PMN<sup>a</sup>

	PMA	FMLP	IgG	STZ
Treated without cytochalasine B				
IgA nephropathy (n = 20)	0.97 ± 0.27	1.62 ± 0.53*	0.34 ± 0.12	1.05 ± 0.22
Normal controls (n = 20)	0.99 ± 0.35	2.03 ± 0.83*	0.28 ± 0.09	1.09 ± 0.23
Treated with cytochalasine B				
IgA nephropathy (n = 20)	1.08 ± 0.39	3.56 ± 0.66	0.65 ± 0.17*	1.57 ± 0.29*
Normal controls (n = 20)	1.02 ± 0.33	3.31 ± 0.64	0.53 ± 0.15*	1.31 ± 0.34*

<sup>a</sup>Mean ± 1 SD. PMA, phorbol myristate acetate; FMLP, n-formyl-methionyl-leucyl-phenylalanine; IgG, heat-aggregated human IgG; STZ, serum-treated zymosan.

\*P < 0.05.

also not significantly different from that in normal controls (Table 2, Fig. 1). The H<sub>2</sub>O<sub>2</sub> production by PMN after such stimulations in patients with grades III and IV stage IgA nephropathy was slightly higher than that in patients with grades I and II stage IgA nephropathy, but there was no statistical significance ( $P > 0.05$ ). There were no statistical correlations between the H<sub>2</sub>O<sub>2</sub> production by PMN after stimulation with any of the four stimulants and the levels of BUN, s-creatinine, CCR, and PSP 15 min.

#### Glomerular Infiltration of PMN

Although glomerular infiltration of PMN was not observed in patients with grade I stage IgA nephropathy, it was observed in one out of five patients (20.0%) with grade II stage (Table 3). Glomerular infiltration of PMN was observed in six out of eight patients (75.0%) with grade III stage IgA nephropathy, and in one patient (100.0%) with grade IV stage (Table 3). The number of PMN was one to five per glomerulus, while in normal glomeruli, the PMN staining was essentially negative (Fig. 1a,b). No staining of PMN was observed in the tubulo-interstitial regions in patients with IgA nephropathy and normal renal tissues (Table 3).

#### DISCUSSION

Several investigators have indicated the pathogenetic, developmental, and/or exacerbating factors for patients with IgA nephropathy. However, the effects of reactive oxygen species in response to immunological stimulation in patients with IgA nephropathy are still unknown. Recently, Kincaid-Smith et al. (14) reported that infiltration of PMN in glomeruli was observed in specimens from patients with IgA nephropathy taken within 30 days after attacks of macroscopic hematuria, which was thought to be the highest relative risk for progression of renal injuries. In the present study, PMN were clearly observed in the glomeruli of patients with moderate (grade III) and advanced (grade IV) stage IgA nephropathy by immunofluorescence. The infiltration of PMN can be considered one of the major factors behind progression of renal injuries because of its high potential for producing reactive oxygen species.

IgA nephropathy is now generally recognized as immune-complex mediated glomerulonephritis using the immunohistochemical analyses (10). The presence of circulating IgA-dominant immune-complexes in sera was well known in patients with IgA nephropathy. A recent study by Tao et al. (15) revealed an enhanced expression of C3 receptors on PMN in patients with IgA nephropathy. Using heat-aggregated IgG as stimulant, PMN from patients with IgA nephropathy showed higher H<sub>2</sub>O<sub>2</sub> production after further enhancement by cytochalasine B. The same results were obtained after STZ stimulation in this study. The increased oxidative metabolic response of PMN to immunological stimulants in patients with IgA nephropathy further supports the important role of reactive oxygen species in the progression of this disease. It appeared that the oxidative metabolic response of PMN might be stimulated by increased circulating immune-complex levels in patients with IgA nephropathy. Recently, Eguchi et al. (16) and Rauterberg et al. (17) reported a marked deposition of poly C9 (MAC) in glomeruli and extraglomerular vascular walls in some patients with IgA nephropathy. It is postulated that the presence of poly C9 in renal tissues is another indication of renal injury since poly C9 is also a powerful stimulant to cultured mesangial cells that produce reactive oxygen species as described by Adler et al. (18). Using PMA as a classical stimulant, PMN in the peripheral blood obtained from patients with IgA nephropathy showed normal H<sub>2</sub>O<sub>2</sub> production in this study. The same results were obtained from hemodialysis patients (6,8), which indicates that non-specific stimulation, as with the PMA model, may not induce abnormal oxidative metabolism of PMN in patients with renal diseases. The lower rate for FMLP-stimulated H<sub>2</sub>O<sub>2</sub> release in patients with IgA nephropathy was the same as in the study of dialysis patients (8). FMLP is the most widely studied synthetic formylated peptide for activating chemoattractant receptors of PMN. Thus, it is postulated that patients with IgA nephropathy already have deficient chemotactic receptor-mediated response of PMN although their renal functions remain normal. Further examination is warranted to determine the relationship between the H<sub>2</sub>O<sub>2</sub> production by PMN after the stimulation and the degree of the PMN infiltration in glomeruli of patients with IgA nephropathy.

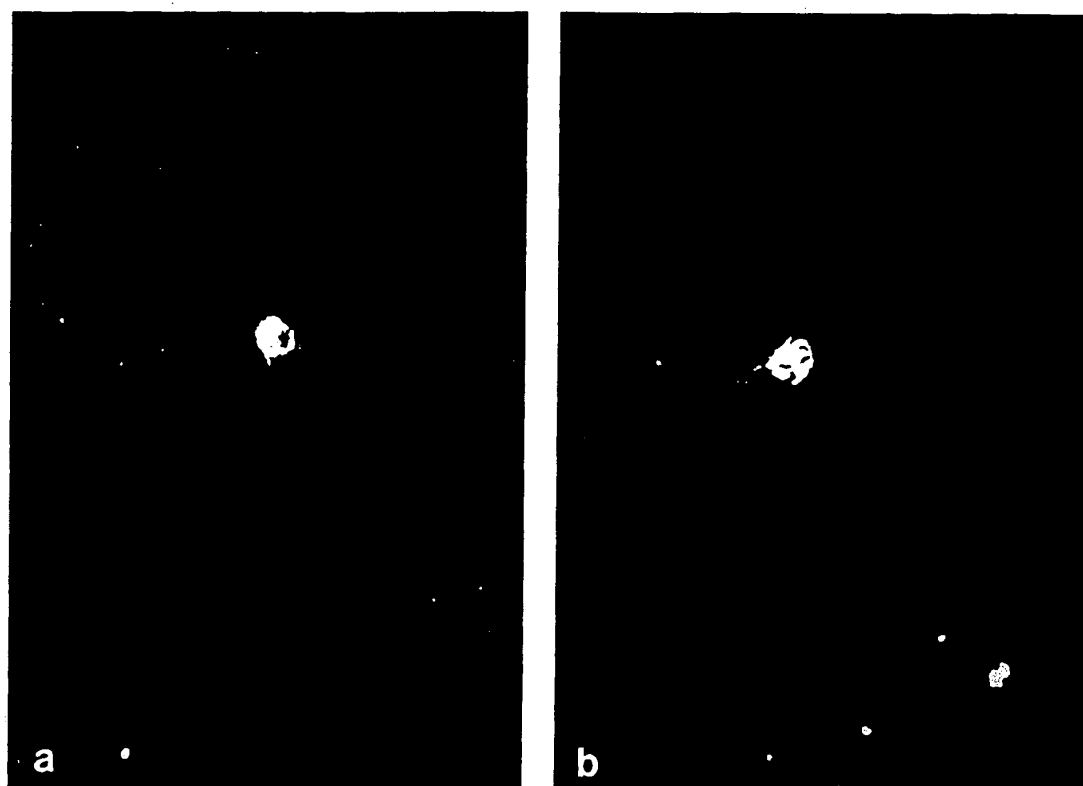


Fig. 1. Glomerular infiltration of PMN as demonstrated by monoclonal anti-PMN antibody in a patient with IgA nephropathy.  $\times 400$ .

Neutrophils and monocyte-macrophages are considered to produce high quantities of reactive oxygen species in peripheral blood and various tissues. Several studies in patients with different types of proliferative glomerulonephritis including IgA nephropathy have confirmed glomerular monocyte infiltration (19,20). In 1989, Tomino et al. also reported that the T cell subsets (OKT4, OKT8) and monocytes and null cells (OKM1) in glomeruli from patients with IgA nephropathy were significantly increased as compared to those from patients with mesangial proliferative glomerulonephritis (non-IgA

nephropathy) (21). It appears that the infiltration of macrophages and PMN in glomeruli might induce the reactive oxygen species and then initiate the glomerular injuries in patients with IgA nephropathy.

It is concluded that reactive oxygen species produced by PMN after stimulation under various conditions may play an important role in progression and exacerbation in patients with IgA nephropathy.

#### ACKNOWLEDGMENTS

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TABLE 3. Incidence of PMN Infiltration in the Glomeruli of Patients With IgA Nephropathy and Normal Tissues by Immunofluorescence

Disease	Incidence of PMN in glomeruli
IgA nephropathy	
Grade I	0/3 (0.0%)
Grade II	1/5 (20.0%)
Grade III	6/8 (75.0%)
Grade IV	1/1 (100.0%)
Normal renal tissues	0/5 (0.0%)

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# Superoxide Dismutase in Diabetic Polymorphonuclear Leukocytes

N. NATH, S. N. CHARI, AND A. B. RATHI

## SUMMARY

The level of superoxide anion was found to be significantly elevated in polymorphonuclear leukocytes (PMNL) from diabetic subjects as compared with those from normal subjects. This elevation was attributed to the significant reduction in the activities of both cytoplasmic and mitochondrial superoxide dismutase (SOD), the effect being more pronounced in the cytoplasmic fraction. Although the content of copper decreased considerably in the diabetic PMNL, the decrease in the zinc content was less significant, with an insignificant alteration in the content of manganese. PMNL obtained from insulin-treated diabetic patients showed considerable alleviation of SOD levels. The implication of these results are discussed herein. *DIABETES* 33:586-589, June 1984.

**T**he activity relationship of superoxide dismutase (SOD) in various pathologic conditions is not clear, since study attempts in this direction have been few. Although the activity of this oxygen-detoxifying metalloenzyme was found to vary from normal in various human leukemic cells,<sup>1</sup> very few reports are available from diabetes studies. In vivo studies and experiments with isolated pancreatic islets have indicated that the diabetogenic action of alloxan is mediated by the hydroxyl radical generated via reactions that involve superoxide anion,  $H_2O_2$ , and iron.<sup>2,3</sup> This phenomenon was supported by the fact that prior administration of SOD did afford protection against streptozotocin-induced diabetes.<sup>4</sup>

Under pathologic conditions, any alteration in the activity of SOD could well be represented in the blood cells, since they are in constant contact with molecular oxygen. Although

significant lowering in the activity of erythrocytic SOD has been demonstrated in experimental diabetes induced by streptozotocin,<sup>5</sup> information is lacking about the activity of this enzyme in human diabetic PMNL.

We report herein alterations in the content of superoxide and the activity of SOD in diabetic PMNL. The contents of copper, zinc, and manganese were also measured as a requisite determinant for the activity of SOD.

## MATERIALS AND METHODS

**Materials.** Dextran (mol wt 150,000-200,000) was obtained from B.D.H. Chemicals, United Kingdom; ficoll-hypaque from Decruz Corporation, Bombay, India; cytochrome-C from V.P. Chest Institute, New Delhi, India; and xanthine oxidase (buttermilk) and superoxide dismutase (bovine blood) from Sigma Chemicals, St. Louis, Missouri. All other chemicals and reagents used were of analytic grade.

**Subjects.** Male subjects (40-55 yr) admitted to the Government Medical College and Hospital, Nagpur, were chosen for the study. Two groups of diabetic subjects were studied, the first group receiving insulin and the second group not receiving insulin treatment. Initially, a complete history, physical examination, and baseline laboratory assessment were obtained. Baseline laboratory tests are shown in Table 1. The diabetic patients had positive glucose tolerance tests; hence, they were truly diabetic and not merely hyperglycemic. The nonketotic diabetic group consisted of patients with newly diagnosed diabetes with postprandial blood sugar levels of 250-450 mg/dl with 1.5-2 g/dl sugar in urine. The 8 treated diabetic patients were those who had received insulin for the past 5-7 yr and had postprandial blood sugar levels of 110-170 mg/dl without any sugar in urine. During the period of hospitalization, all patients received a diet composed of 40% carbohydrate, 35% fat, and 22% protein. Care was taken to choose 8 healthy, nondiabetic controls of similar age groups receiving a more or less similar caloric percentage in their diet. None of the above groups were underweight or malnourished, obese, or had any renal or other complication (or infection) at the time of study.

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TABLE 1  
Baseline laboratory findings in diabetic subjects

Patient number* (all males)	Age (yr)	Body wt (kg)	Height (cm)	Serum albumin (g/dl)	Serum bilirubin (mg/dl)	Serum creatinine (mg/dl)	Blood urea (mg/dl)	Blood Hb (g/dl)	TLC
1	45	74	183	4.0	0.7	1.2	31.4	12.5	6600
2	42	73	180	4.2	0.8	1.4	38.2	13.6	6800
3	50	62	174	4.1	1.0	1.8	32.8	14.2	7200
4	53	69	176	3.5	0.8	1.0	40.1	14.4	6900
5	42	65	177	4.0	0.7	2.1	28.2	14.0	7800
6	49	74	179	3.9	1.1	2.0	35.4	15.0	6600
7	51	77	182	3.8	0.9	1.6	26.8	14.8	7500
8	50	71	185	4.4	1.1	1.4	32.1	13.2	7800
9	55	69	178	4.2	0.7	1.5	30.8	14.4	6600
10	46	67	177	3.9	0.6	1.4	29.0	14.2	6200
11	51	72	182	4.2	0.5	1.5	26.2	15.2	7100
12	50	68	181	3.5	0.7	1.8	30.1	14.8	7200
13	48	70	176	4.2	0.9	2.1	25.9	13.8	6900
14	45	72	184	3.8	0.8	1.4	30.8	14.4	6500
15	53	72	179	3.7	1.1	1.6	31.2	14.8	6600
16	49	71	178	3.8	0.8	1.8	32.4	14.6	6800
Control	45-55	65-75	170-180	3.5-5.0	0.4-1.0	1-2	21.2-38.4	13.2-15.4	6800-7200

\*Patients 1-8 were diabetic and not receiving insulin; patients 9-16 were diabetic and treated with insulin.

**Isolation of PMNL.** Heparinized, fasting blood samples were drawn from control and diabetic subjects after one day of hospitalization and PMNL separated immediately by the dextran sedimentation method of Boyum,<sup>6</sup> with slight modifications. Blood was allowed to stand for 45 min at 20-25°C, the plasma was aspirated carefully, and centrifuged at 170 × g for 10 min. Pellets were then suspended in Hanks' balanced salt solution (with glucose) and placed on top of a ficoll-hypaque mixture and centrifuged at 400 × g for 40 min. Pellets thus obtained were washed with 0.34 M sucrose to remove platelets. A few remaining erythrocytes were disrupted by hypotonic lysis with cold distilled water for 30 s. Isotonicity was restored by 3.5% NaCl. PMNL were finally washed and suspended very slowly in 0.34 M sucrose. This solution contained 99% PMNL as ascertained by phase-contrast microscopy. The entire operation was carried out in the cold (0-4°C).

**Enzyme assay.** The PMNL pellet was suspended in Hanks' balanced salt solution (HBSS) and sonicated for 1 min using a Ralsonics Ultrasonic Processor (with controls set at 140)

and centrifuged at 800 × g for 10 min to remove nuclei and unbroken cells. The supernatant was centrifuged at 10,000 × g for 15 min to obtain the mitochondrial pellet. The 10,000 × g supernatant was further spun at 15,000 × g for 20 min, which resulted in a clear cytoplasmic supernatant. The mitochondrial pellet was resuspended in 1 ml HBSS and treated with 0.2% triton X-100 to lyse the mitochondria, and was again sonicated and centrifuged at 10,000 × g for 20 min. SOD activity was then assayed in both of the clear supernatants by the method described by Ross et al.<sup>7</sup>

The clear supernatant (0.2 ml) was added to a mixture of 20 mM Na<sub>2</sub>CO<sub>3</sub>, 0.1 mM EDTA, 5 μM cytochrome-C, 50 μM xanthine, and 1 mM NaN<sub>3</sub> (pH 10) and incubated for 5 min at 25°C. The reaction was started by the addition of xanthine oxidase (final concentration 0.01 U/ml), and the final volume was 2 ml. The increase in absorbance at 550 nm was measured for 10 min against a blank vial without cell extract.

One unit of activity is defined as the amount of enzyme required to inhibit a standard rate of ferricytochrome-C reduction by 50%.

TABLE 2  
Production of superoxide and activity of superoxide dismutase in normal and diabetic polymorphonuclear leukocytes (mean ± SD)

Groups	Superoxide produced (nmol cytochrome-C reduced/mg protein/15 min)	Superoxide dismutase (U/mg protein)	
		Cytoplasmic	Mitochondrial
Control (8)*	1.51 ± 0.56	3.43 ± 0.75	0.76 ± 0.15
Diabetic, nonketotic (8)	2.98 ± 0.50	1.80 ± 0.87	0.55 ± 0.10
†P	< 0.001	< 0.01	< 0.02
Diabetic, insulin-treated (8)	1.94 ± 0.48	3.01 ± 0.90	0.74 ± 0.16
‡P	< 0.01	< 0.05	< 0.05

\*Number of subjects is shown in parentheses.

†P as compared with control subjects.

‡P as compared with diabetic, nonketotic subjects.

## DIABETIC PMNL SOD

TABLE 3

Contents of zinc, copper, and manganese in normal and diabetic polymorphonuclear leukocytes (mcg/kg dry wt, mean  $\pm$  SD)

Groups	Zinc	Copper	Manganese
Controls (8)*	25.34 $\pm$ 4.12	11.74 $\pm$ 2.44	5.22 $\pm$ 1.44
Diabetic, nonketotic (8)	19.12 $\pm$ 4.04	6.84 $\pm$ 2.58	5.34 $\pm$ 1.12
P†	< 0.02	< 0.01	NS
Diabetic, insulin-treated (8)	23.82 $\pm$ 4.14	10.14 $\pm$ 2.89	5.28 $\pm$ 1.64
P‡	< 0.05	< 0.05	NS

\*Number of subjects is shown in parentheses.

†P as compared with control subjects.

‡P as compared with diabetic, nonketotic subjects.

NS, not significant.

**Measurement of superoxide.** The production of superoxide was measured by the method described by Johnston et al.<sup>8</sup> Sonicated PMNL cells were suspended in 1 ml HBSS and preincubated for 5 min at 37°C. SOD (30  $\mu$ g) was added in a final volume of 1.3 ml. Ferricytochrome-C (0.1 ml, 1.2 mM) was added to begin the reaction. The mixture was incubated for 10 min, the reaction was stopped by ice bath, centrifuged at 200  $\times$  g for 10 min at 4°C, and absorbance of the supernatant measured at 550 nm. Results were converted to nmol cytochrome-C reduced using the extinction coefficient  $E_{550\text{ nm}} = 2.1 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ . Tubes containing only buffer and cytochrome-C were incubated as above and served as blanks.

**Measurement of zinc, copper, and manganese.** PMNL Zn, Cu, and Mn were extracted by the method of Parker et al.<sup>9</sup> and contents determined using an atomic absorption spectrophotometer (Shandon Southern A-3400).

Protein was determined by the method of Lowry et al.<sup>10</sup> using bovine serum albumin as standard.

## RESULTS

It is evident from Table 2 that the activities of both cytoplasmic and mitochondrial SOD were decreased in the diabetic PMNL as compared with those from normal subjects, the effect being more pronounced in the cytoplasmic fraction ( $P < 0.01$ ). Consequently, the level of superoxide was found to be increased significantly in the diabetic PMNL ( $P < 0.001$ ). Considerable alleviation of the SOD level (>75%) was observed in PMNL obtained from insulin-treated diabetic patients.

The decrease in the level of copper in diabetic PMNL was found to be more significant ( $P < 0.01$ ) than that of zinc ( $P < 0.02$ ), with no change in the contents of manganese (Table 3). PMNL from insulin-treated diabetic patients showed considerable alleviation (>75%) in the contents of zinc and copper.

## DISCUSSION

The presence, within human PMNL, of two commonly found eukaryotic SOD in two distinct subcellular locations, namely the mitochondria and cytoplasm, has been well established.<sup>11</sup> For the first time, evidence is presented of a decrease in the activities of both dismutases with a concomitant increase in the content of superoxide free radical in diabetic PMNL. Observed reduction in the contents of copper and

zinc in diabetic PMNL could contribute to the depressed activity of cytosolic SOD, known to be dependent on these metal ions;<sup>11</sup> however, no reason could be rendered for such a decrease in the leukocytic copper and zinc in diabetes. However, earlier works have demonstrated elevated serum copper<sup>12</sup> and an unexplained hyperzincuria<sup>13</sup> in this metabolic disease.

The implication of decreased zinc content in diabetic PMNL could be of some interest. If leukocytes were deprived of  $\text{Zn}^{2+}$ , which is known to preserve the integrity of the cell membrane by interacting with membrane thiol groups to form zinc mercaptide,<sup>14</sup> such biochemical alterations as decrease in sialic acid,<sup>15</sup> reduced glutathione (GSH),<sup>16</sup> and activity of membrane-integrated enzymes<sup>17</sup> could occur, leading to the functional aberration of PMNL so commonly observed in diabetes.<sup>18</sup>

The decrease observed in the activity of mitochondrial SOD (Mn-dependent) of diabetic PMNL is difficult to explain in view of our failure to observe any decrease in the content of leukocytic manganese. However, our recent observation of decreased GSH in diabetic PMNL<sup>16</sup> could in part explain for the decreased activity of this sulfhydryl enzyme.

The alleviation observed in the alterations of superoxide, SOD, and metal ions in PMNL obtained from diabetic patients only indicates that the changes are an effect of relative insufficiency of insulin, rather than being the cause of the disease.

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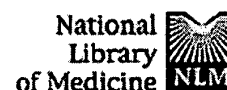
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## Roles of advanced glycation end-products in the progression diabetic nephropathy.

Makino H, Shikata K, Kushi M, Hironaka K, Yamasaki Y, Sugimoto H, Ota Z, Araki N, Horiuchi S.

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Available data indicate that the development of diabetic nephropathy is linked to hyperglycaemia. Glucose reacts nonenzymatically with proteins form Schiff base and Amadori products. Further incubation of these early products leads to the formation of advanced glycation end-products (AGEs). AGEs seem to play a central role in the progression of diabetic nephropathy. Immunohistochemically, AGEs were also detected in an expanded mesangial matrix, especially in nodular lesions from patients with diabetic nephropathy. AGEs staining was noted in the Bowman's capsule, periglomerular fibrosis and sclerosing glomeruli. In our ultrastructural study of mesangial matrix from patients with diabetic nephropathy by high-resolution scanning electron microscopy after cellular removal, the meshwork structure was evident at higher magnification. In nodular lesions, the loose meshwork structure appeared to be composed of various sized strands, ranging from 6 to 24 nm (mean +/- SD: 11.4 +/- 3.8 nm). The pore sizes were variable, ranging from 10 to 70 nm (mean +/- SD: 23.6 +/- 12.3 nm), and were statistically larger than those of normal controls. As the AGEs are localized most notably in nodular lesions, advanced glycation end-products play a role in the progression of diabetic nephropathy through impairment of the assembly of matrix proteins in vivo. Because type V and type VI collagens are the major components of nodular lesions, increases in these interstitial and fibrillar or microfibrillar collagens may contribute to the formation of wider strands in the mesangial matrix of a nodular lesion. As no metalloprotease that is specific for type VI collagen has been identified thus far, AGEs formation might occur preferentially in type VI collagen-rich nodular lesions, which are sites of slow turnover.

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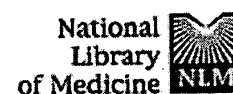
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### Quantification of glomerular TGF-beta 1 mRNA in patients with diabetes mellitus.

Iwano M, Kubo A, Nishino T, Sato H, Nishioka H, Akai Y, Kurioka I, Fujii Y, Kanauchi M, Shiiki H, Dohi K.

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Transforming growth factor-beta 1 (TGF-beta 1) is a primary determinant of the mesangial expansion observed in diabetic nephropathy. In this study, we quantitated the levels of intraglomerular TGF-beta 1 mRNA in patients with diabetes mellitus using a competitive polymerase chain reaction (PCR) method. Renal biopsy specimens were obtained from 29 patients with non-insulin-dependent diabetes mellitus. Total RNA was extracted from the glomeruli and reverse transcribed into cDNA with reverse transcriptase. We prepared samples containing identical amounts of beta-actin cDNA (8 pg), performed competitive PCR by co-amplifying mutant templates of beta-actin with a unique EcoRI site. We also used this competitive PCR method to measure TGF-beta 1 cDNA by co-amplifying mutant templates of TGF-beta 1. We observed higher expression of TGF-beta 1 mRNA in glomeruli of patients with diabetic nephropathy as compared with normal glomeruli. Intraglomerular TGF-beta 1 mRNA was elevated, even in the early stage of diabetic nephropathy. Moreover, levels of intraglomerular TGF-beta 1 mRNA correlated with values of HbA1c. These data suggest that hyperglycemia induces intraglomerular TGF-beta 1 mRNA expression in vivo, and that TGF-beta 1 overproduction may be associated with the progression of diabetic nephropathy.

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## REVIEW

# RECENT ADVANCES IN DIABETIC NEPHROPATHY : HOW BIG A CULPRIT IS GLUCOSE ?

G.E. STRIKER, L.J. STRIKER

**A**lthough the renal complications of diabetes mellitus are multifactorial and influenced by various factors such as systemic hypertension or hyperlipaemia, the role of hyperglycaemia has recently been especially emphasized by clinical trials and experimental data. In this essay we will review some of the more recent therapeutic trials as well as experimental data from our own laboratory suggesting that advanced glycosylation end-products (AGEs) play a role in the development and progression of diabetic nephropathy.

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in the United States and in many other developed countries [1]. The incidence and prevalence of ESRD in patients with insulin-dependent diabetes mellitus (IDDM) vary among racial groups, being more common in Caucasians than in African Americans, Asians, Hispanics, or native Americans [2]. The opposite is true for end-stage renal disease due to non-insulin-dependent diabetes mellitus (NIDDM). The rates for each region of the United States and the world appear to be directly related to the incidence and prevalence of IDDM or NIDDM [3].

The factor(s) leading to the initiation of kidney disease in the susceptible diabetic patient, and those that determine its subsequent rate of progression to end-stage kidney failure, have not been completely elucidated, but the control of glycaemia and hypertension are quite important ones. The response to close metabolic control in IDDM patients has been a subject of considerable controversy. Many early studies included too few patients, and conflicting conclusions were drawn about the effect of close metabolic con-

trol, which remained as a major point of controversy since close control carries the risk of hypoglycaemic complications. An analysis of the Steno studies showed that only those participants in intensive treatment cohorts who had an albumin excretion rate (AER) of > 100 mg/day had an increased chance of progressing to an AER of > 300 mg/day [4]. A study of 213 patients who developed IDDM before the age of 15, between the years 1961 and 1980, and who were followed from diabetes onset to 1991, revealed that the cumulative incidence of persistent albuminuria (dip-stick positive) decreased from 30 to 5.8 % [5]. Importantly, the mean glycosylated haemoglobin value was 7.0 % in the latest time period studied, and was higher in diabetic patients with persistent proteinuria as compared to those with no albuminuria. This study of a homogeneous population suggests that glucose control exerts an effect separate from that due to a genetic propensity for renal disease, a postulate which has had considerable support [6].

Prospective, randomised studies addressing the issues of close metabolic control and blood pressure control are the subject of this section. NIDDM will not be considered, except in the recommendations section. Although many physicians feel that data from trials dealing with IDDM may be of relevance to the NIDDM patient, this opinion awaits confirmation by clinical trials.

### Definition of diabetic nephropathy

The definition of diabetic nephropathy has undergone considerable modification. The most reliable method to make the diagnosis is histologic, but this is not an accepted practice in most cases. Thus, alternate methods such as albumin excretion rate (AER) have been proposed as a surrogate by many groups. More than 90 % of patients with dipstick-positive proteinuria (> 300 mg/day) progress to ESRD [1]. This fact led to studies of lesser degrees of albumin leak into the urine, so-called "microalbuminuria". This

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term does not refer to the size of the albumin molecules, but to the amount of albumin in the urine. The upper limit of normal is an AER of approximately 40 mg/day, but the actual risk of developing permanent albuminuria and progressive renal disease has been most clearly established for those with an AER of  $\geq 100$  mg/day. Most studies of the early stages of diabetic nephropathy are based on analyses of several levels of microalbuminuria. However, levels of microalbuminuria (especially lower ones) may show considerable variation.

### Frequency

Few studies have addressed the issue of the proportion of IDDM or NIDDM patients who develop kidney disease. Since microalbuminuria is a well-defined risk factor, many studies have utilised this factor as the criteria for screening IDDM patients. One of the earliest studies showed that an elevated AER (20–200  $\mu\text{g}/\text{min}$ ) occurred in nearly 22 % of Danish patients with a diabetes duration of  $> 5$  years [7]. A large group (1,888 participants) of non-hypertensive IDDM patients attending hospital clinics in England were screened, and it was found that the incidence of AER  $> 30$ –250  $\mu\text{g}/\text{min}$  in an overnight urine sample was 3.7 % [8]. This difference was probably due to the inclusion of patients with a diabetes duration of  $< 5$  years and the exclusion of hypertensives. Nonetheless, these studies on two different Caucasian populations of IDDM patients established the fact that an elevated AER was present in a significant number of IDDM patients and served as the basis for subsequent natural history and intervention studies on diabetic nephropathy.

## ■ THE ROLE OF GLUCOSE CONTROL IN DIABETIC NEPHROPATHY: THE DIABETES CONTROL AND COMPLICATIONS TRIAL (DCCT)

### Background

Studies in experimental animals have clearly shown that close glycaemic control can prevent the development of proteinuria and nephropathy, but similar results have not been convincingly demonstrated in man. Accordingly, the DCCT was undertaken to address this question in the IDDM patient [9]. The DCCT was a randomised clinical trial comparing conventional diabetes management with intensive treatment designed to achieve glucose blood levels as close to normal as possible. Two separate questions were addressed: 1) Does intensive treatment prevent or delay the development of complications in patients who had no complications during baseline observations (the primary prevention cohort)? and 2) Does

intensive treatment prevent or slow the progression of complications in patients who had evidence of complications during the baseline period (the secondary intervention cohort)?

### Description of the trial

**Baseline Characteristics** – DCCT participants ranged from 13 to 39 years of age at randomisation, had IDDM duration of 1 to 15 years, had experienced no advanced micro- or macrovascular diabetic complications, had normal renal function, and were all normotensive (BP  $< 140/90$  mm Hg). Ninety-six percent were Caucasian, and approximately 50 % were female. For the 725 subjects in the primary prevention cohort, IDDM duration ranged between 1 and 5 years. They had no detectable retinopathy, a urinary AER of  $< 28$   $\mu\text{g}/\text{min}$ , and serum C-peptide levels of  $< 0.5$  pmol/ml after stimulation. The 715 participants in the secondary intervention cohort had a diabetes duration of 1 to 15 years, minimal to moderate retinopathy, and an AER  $< 139$   $\mu\text{g}/\text{min}$ . Overall, there were no other significant differences between the two groups at baseline. Two hundred and seventy-eight participants were followed for 9 years, and most of the other 1,163 patients were followed for at least 4 years.

**Randomisation and Treatment Plan** – Conventional treatment: The participants received 1 or 2 daily injections of insulin consisting of a mixture of intermediate and long-acting insulin, which was not usually adjusted on a daily basis, although urine and blood glucose were measured daily. The therapeutic goals were the absence of hyperglycaemia, hypoglycaemia, or ketonuria, and the maintenance of normal growth and development as well as body weight. In addition to the care received by the conventional therapy group, the intensive treatment group measured their glycaemia at least 4 times during the day (and once a week at 3 AM), and were required to adjust their insulin dosage to keep blood glucose between 70 and 120 mg/dl.

**Renal Function Measurements** – Specimens for urinalysis, urine culture (for all females), AER, creatinine clearance, and plasma creatinine and albumin were collected at annual visits. GFR was estimated by  $C_{cr}$  annually. GFR was measured by  $^{125}\text{I}$  insulin clearance at 3 years and study termination, using techniques validated in the United States for the modification of diet in renal disease (MDRD study) [10].

### Results

**Overall Results** – Mean follow-up was 6.5 years (range 3–9). Data were collected for 99 % of the total study population. Adherence to randomly assigned treatment group criteria was  $> 97$  % in both groups, as

verified by the fact that at baseline mean HbA<sub>1c</sub> levels did not differ between treatment groups. At the end of the study, the values were 7.2 % vs. 9.1 %, ( $P < 0.001$ ) for intensive vs. conventional treatment groups respectively. At the end of the study, mortality did not significantly differ between the groups. The incidence of severe hypoglycaemia was increased three-fold in the intensive treatment group (62/100 participant years vs. 19/100 in the conventional treatment group); and there were no deaths, myocardial infarctions, or strokes attributable to hypoglycaemia. Nor was there any significant difference between the two groups in the number of major accidents requiring hospitalisation. However, in the intensive treatment group there was an increase of 33 % in the mean adjusted risk of reaching more than 120 % of ideal body weight.

### Renal Outcome

**Intention to treat analysis** – Fewer participants in the intensive treatment group, in either cohort, developed microalbuminuria ( $> 40$  mg/24 h) or albuminuria ( $> 300$  mg/24 h) [9]. There was a 34 % reduction of the mean adjusted risk of microalbuminuria in the primary-intervention cohort and of 56 % in the secondary-intervention cohort in the intensive therapy group. However, only 2 patients in the primary-intervention group and 5 in the secondary-intervention group developed albuminuria ( $> 300$  mg/24 h) or a C<sub>cr</sub> of  $< 70$  ml/min/1.73 m<sup>2</sup> in the overall cohort.

### Secondary analyses of renal results

A detailed analysis of DCCT renal function results has recently been published [11].

- AER  $> 28$  µg/min – In the primary prevention cohort 41/346 of those in the intensive treatment group and 67/378 in the conventional therapy group developed microalbuminuria of  $> 28$  µg/min. Thus, the cumulative incidence of microalbuminuria over 9 years was 16 % in the intensive treatment group and 27 % in the conventional therapy group, representing a 34 % reduction in risk ( $p = 0.04$ ). Importantly, the risk gradually increased over time in the conventional treatment group (from 2.7–4.8 cases/100 person-years), whereas it decreased (3.2–1.6 cases/100 person-years) in the intensive therapy group.

Microalbuminuria of  $> 28$  µg/min developed in 26 % of the intensive treatment group of the secondary prevention cohort after 9 years, and in 42 % of the conventional therapy group, indicating a 43 % reduction in risk ( $P < 0.0001$ ). In contrast to results for the primary prevention cohort, the rate of developing microalbuminuria fell in both the intensive and conventional treatment groups over the 9 years of follow-up, and the differences between the groups remained stable.

In the combined cohorts, there was a 39 % reduction in relative risk for microalbuminuria in the intensive therapy group ( $P < 0.0001$ ).

- AER of  $> 70$  µg/min (in patients with a baseline level  $< 28$  µg/min) – Within the primary prevention cohort, 10 patients from the intensive treatment group and 18 from the conventional therapy group developed an AER of  $> 70$  µg/min, for an overall incidence of 7.0 and 20 % respectively ( $P < 0.002$ ), and a mean reduction in absolute risk of 56 % in the intensive treatment group.

Within the secondary intervention cohort, 32 patients from the intensive treatment group and 43 from the conventional treatment group developed an AER of  $> 70$  µg/min, for a cumulative incidence of 10 % and 20 %, respectively ( $P = 0.002$ ), and a mean reduction in absolute risk of 56 %.

Thirty-two of the 671 patients in the combined intensively treated group developed an AER of  $> 70$  µg/min, as compared to 61/694 in the conventional therapy group, for an absolute risk reduction of 51 %.

Even though the number of participants in each group who reached this AER level was small, the differences between conventional and intensive treatment were significant, and intensive therapy showed a benefit.

- AER of  $> 208$  µg/min – Only 3 intensively-treated and 6 conventionally-treated participants in the primary prevention cohort developed an AER of  $> 208$  µg/min.

Within the secondary intervention cohort, 15/363 patients from the intensive treatment group and 31/357 from the conventional treatment group developed an AER of  $> 208$  µg/min, for cumulative incidence rates respectively of 5.2 and 11.4 % ( $P < 0.01$ ), with a mean risk reduction of 56 % in the intensive treatment group. Although there was a tendency for the risk to decrease over time in the intensive treatment group and increase in the conventional therapy group, the number of cases was small, and the trends were not significant.

### Summary of secondary analyses of AER

**Primary prevention cohort** – Secondary analysis revealed four important points in the intensive therapy group. First, intensive therapy reduced the risk for development of sustained microalbuminuria (either 28 or 70 µg/min) or clinical albuminuria ( $> 208$  µg/min) by 51 % and 67 % respectively. Sustained microalbuminuria is a more stringent test of intensive treatment and may thus be more representative of the true benefits of glucose control than analyses based on a single determination of the AER, since the presence and degree of microalbuminuria may wax and wane, espe-

cially at lower levels of AER. Secondly, while there was a rise in the prevalence of elevated AER in both treatment groups, the increase was more rapid and of greater magnitude in the conventional therapy group. These data show that, while there was an overall risk of an increase in AER in both treatment groups, it was blunted in the intensive therapy group. Thirdly, most of the benefits of intensive therapy relative to the rate of change in AER were achieved within the first year. Somewhat surprisingly, the long-term slopes of the intensive and conventional treatment groups did not differ, and the rates of change in both treatment groups were the same after the first year. However, within the intensive treatment group, those rates in the upper quartile of progression did increase with time, even though the mean rate of progression for the overall group did not. Thus, a subgroup within the overall group could benefit from intensive therapy. The rate of change in the AER increased in the conventional therapy group, both for the overall mean and in the upper quartile. Importantly, 43 % of the intensive treatment group and 45 % of the conventional treatment group showed an increase in the AER rate. There was an increase of 20 %/year in 8 % of the intensive treatment group and 10 % in the conventional treatment group.

Thus, the data show that intensive treatment resulted in decreased risk of elevated AER. However, it is noteworthy that this reflected essentially the 15 % decrease in AER found during the first year in the intensive treatment group.

Finally, it may be noted that a large group of patients in the treatment groups showed progression: 43 % in the intensive treatment group, and 45 % in the conventional treatment group.

**Secondary prevention cohort** – There was no difference between the groups at one year, except for the fact that in the secondary prevention cohort intensive treatment resulted in a -0.25 % increase per year, whereas there was a 6.5 % increase in the AER rate in the conventional therapy group. This resulted in a 56 % reduction in the risk of reaching clinically recognizable proteinuria in the intensive therapy group. There was no significant difference in the slope of AER between the two treatment groups at the end of the study.

#### *Glomerular filtration rate (GFR)*

There were no differences in  $C_{cr}$  or insulin clearance in either cohort between treatment groups at the end of the study (121–125 ml/min/1.73 m<sup>2</sup>). In both cohorts, approximately 35–40 % of the subjects had elevated GFRs (> 130 ml/min/1.73 m<sup>2</sup>) at both 3 and 5 years.

#### *Hypertension*

Hypertension did not differ significantly between cohorts and treatment groups (18 % in conventional treatment groups and 16 % in intensive treatment groups).

#### *Discussion of the DCCT study*

The major renal outcome measurement in this study was the AER. The number of participants who developed changes in the GFR was too small for statistical differences to be apparent. There was an overall reduction in the risk for development of an elevated AER (34 % in the primary prevention group, 39 % in the secondary intervention group, and 39 % for the overall study). The beneficial effect of intensive therapy in the primary prevention cohort was seen in the first year. Thereafter, the rates of change were similar between the intensive and conventional treatment groups. Significantly, 40 % of all participants had positive rates of change in the AER. It is not known whether this apparent beneficial outcome will result in fewer diabetic patients developing ESRD, but it suggests that ESRD would be at least delayed in IDDM patients who receive intensive therapy very early in their course. In the secondary prevention cohort, there were no differences at one year in the treatment groups. However, the AER did not increase in the intensive treatment group, whereas it increased by 6.5 %/yr in the conventional treatment group. Because of the importance of this problem, and the fact that other hard end-points such as blindness and macrovascular disease were not reached during the DCCT, a long-term observational study on the participants is underway.

## ■ THE ROLE OF ADVANCED GLYCOSYLATION IN DIABETIC NEPHROPATHY

### *Introduction*

Glomerular lesions in diabetes mellitus have been extensively characterised immunochemically, and the components of the extracellular matrix (ECM) which accumulate in glomeruli, leading to the obliteration of the tuft, have been recently identified [12–15]. However, the pathogenetic events leading to the lesions have not been elucidated. The accumulation of ECM components in the glomeruli is secondary (at least in part) to metabolic derangements, since glomerular lesions and activation of ECM genes have been prevented by strict glycaemic control in streptozotocin (STZ)-treated diabetic rats [16]. *In vitro* upregulation of type IV collagen, laminin B 1, and fibronectin gene expression have been found in mesangial and endo-

thelial cells cultured in high glucose, suggesting that hyperglycaemia *per se* plays a role in the development of glomerular lesions [17, 18]. There is now considerable evidence to suggest that some of the deleterious effects of glucose occur through the formation of advanced glycosylation end-products (AGEs) which result from the interaction of proteins and glucose [19, 20]. Aldehyde or keto groups of reducing sugars react early with amino acids to form Schiff bases that lead to intermediate products called Amadori early glycosylation products. At later stages, the formation of AGEs results from a series of slow chemical rearrangements producing stable adducts. There are multiple AGEs which are not identified yet. One of their most important properties is that they continue to form cross-links with proteins and to polymerise in the absence of free glucose. This may account for some of the long-lasting effects of AGEs and explain why further deterioration of organ function may take place in the absence of hyperglycaemia.

AGE moieties have been implicated in a series of complications of diabetes and ageing. One of means by which AGEs may contribute to glomerular lesions is by binding to long-lived proteins such as collagens, thereby preventing their normal removal. AGEs may also trap circulating proteins such as IgG or LDL which could contribute to the deposition of multiple proteins in diabetic glomeruli. Recent observations have determined that removal of LDL by the LDL receptor is dramatically impaired when AGEs are bound to lipids [20].

The mechanisms by which AGEs disturb cellular functions are still incompletely understood. AGEs are recognized by polypeptides on the cell surface, and several binding sites have been identified so far [20]. The best characterised receptor known as RAGE (receptor for AGE) is a member of the immunoglobulin superfamily [21-23]. This receptor mediates macrophage/monocyte activation as well as migration. In endothelial cells, occupation of RAGE is followed by activation of the transcription factor NF-kappa B. This leads to overexpression of some cell surface adhesion molecules such as VCAM-1 and may thus be a contributing factor to the pathogenesis of atherosclerosis [21].

AGEs bind to the surface of multiple cells such as macrophages, lymphocytes, and endothelial or mesangial cells. The nature of the intracellular signal following the occupation of the receptor(s) has not been elucidated, but many cells release cytokines following exposure to AGEs. These include IL-1, INF-gamma, TNF $\alpha$ , IGF-1 and PDGF, which have been shown to influence glomerular cell function [20]. AGEs also increase endothelial cell permeability and induce tissue factor production, which may account for alterations of vascular tone in diabetic patients [22].

### Effect of AGEs on glomeruli *in vitro* and *in vivo*

***In vitro* experiments** – Since mesangial expansion plays a major role in the development and progression of diabetic glomerulosclerosis, we sought to examine the response of isolated mesangial cells to AGEs *in vitro*. We found an upregulation of several ECM genes when mouse mesangial cells were plated on AGE-albumin-coated dishes [24]. Type IV collagen, heparan sulfate proteoglycan, and laminin B1 mRNA levels were increased. These increases were mediated by the AGE receptor since they were inhibited when the cells were incubated with AGE in the presence of an antibody against liver AGE-binding proteins.

***In vivo* experiments** – We have recently characterised some of the early glomerular responses to AGEs in normal mice. The coadministration of aminoguanidine, a specific inhibitor of cross-links, with AGE preparations provided the means of assessing the specificity of the glomerular responses. Albumin-derived AGEs were prepared by incubating mouse serum albumin (MSA) with 50 mM glucose-6-phosphate. Unmodified MSA was incubated under the same conditions, but without glucose-6-phosphate, as a control for AGE-modified MSA. Mice received daily injections of AGE-MSA (6 mg/day) or AGE-MSA plus aminoguanidine [AGE-MSA, 6 mg/day; aminoguanidine, 10 mg/day; (AGE+AG)] for 3-4 weeks. Non-injected mice and unmodified MSA-injected mice served as controls.

No lesions were found in injected mice in light microscopy [25]. The amounts of laminin and type IV collagen present in glomeruli as revealed by immunofluorescence microscopy appeared identical in all groups. There were traces of IgG and IgM in mesangial spaces at 3 but not 4 weeks in both AGE and AGE-AG mice. AGEs were detectable in the mesangial areas of AGE-MSA mice by immunofluorescence microscopy and were markedly reduced in AGE+AG mice. This was consistent with an increase in AGEs bound to kidney collagen in AGE-MSA mice, as compared to AGE+AG mice, when measured by an ELISA assay.

Since one of the early events in diabetic nephropathy is glomerular hypertrophy, we measured glomerular volume using a computer-assisted programme [5, 26] and found that the glomerular volume/body weight ratio was increased 39 % in AGE-MSA mice. However, AGE-MSA-injected mice cotreated with aminoguanidine had a significantly smaller increase in glomerular volume. The relative cell number per glomerulus was also determined by counting the nuclei of 50 successive glomeruli. No differences were found between any of the groups.

Since mouse glomeruli must be isolated by microdissection, a competitive PCR method developed in our laboratory, which is sufficiently sensitive to measure cDNA levels in small samples, was used [27-30].



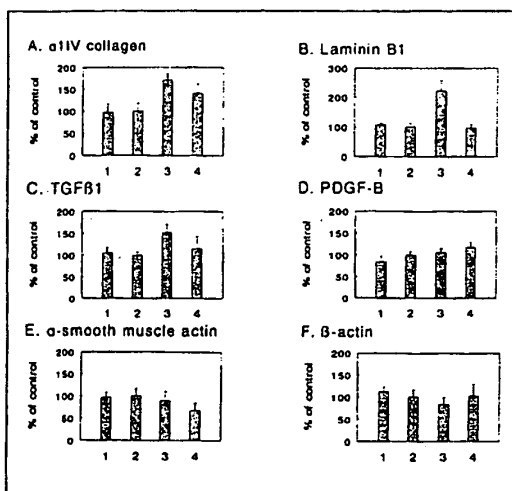


FIG. 1. Representative competitive polymerase chain reaction analysis.

The template cDNAs for all PCR reactions were obtained by reverse transcription *in situ* of pooled microdissected glomeruli. The material in each PCR tube was equivalent to 1/10th of a glomerulus. Decreasing amounts of mutant templates were added to tubes containing the test wild type cDNA. Mutants were constructed by creating new restriction sites ( $\alpha$ 1IV collagen) or deletions (laminin B1, TGF $\beta$ 1  $\alpha$ -SMA,  $\beta$ -actin) that were separated from PCR products by 4% agarose gel electrophoresis. The negative films of the ethidium bromide-stained amplified bands were scanned by laser densitometry. The ratio of mutant to test wild type cDNA band density was calculated for each lane and plotted as a function of the amount of initial mutant template. Glomerular laminin B1 mRNA was increased 2.2-fold in the AGE-MSA mice, whereas aminoguanidine abrogated the laminin B1 mRNA increase in AGE-MSA mice (AGE-MSA vs NORMAL,  $p < 0.01$ ) (Fig. 1).

The  $\alpha$ 1IV collagen mRNA levels were increased 1.7-fold in AGE-MSA mice and 1.4-fold in AGE+AG mice. These results did not differ significantly from those for MSA or NORMAL mice. Levels of  $\alpha$ -smooth muscle actin as well as  $\beta$ -actin mRNA were similar in all four groups. Alpha I type I collagen was not detected in any group.

#### Discussion of the role of ages in diabetic nephropathy

Several observations suggest that ECM gene expression is upregulated during the early phase of diabetic nephropathy [5, 30, 31]. Analysis of total kidney RNA from STZ diabetic rats revealed that laminin

B1 mRNA was increased [32]. Similarly, both laminin B1 and type IV collagen mRNAs were upregulated in STZ diabetic rat glomeruli [16], although these changes were abrogated when the glucose level was reduced by insulin treatment. Since glucose control reduces the rate of formation and the abundance of AGEs, it has been difficult to separate the pathologic effects of hyperglycaemia from those of AGEs. This has been partly achieved through the use of aminoguanidine, an inhibitor of AGE formation [20]. This drug, a small hydrazine-like compound, does not prevent the formation of the early products of non-enzymatic sugar-protein reaction (the Amadori adducts) but reacts with their fragmentation products, thus preventing subsequent rearrangements. Therefore, it inhibits the formation of reactive AGEs and prevents protein cross-linking. As glomerular lesions were markedly reduced in aminoguanidine-treated diabetic STZ rats [33], AGEs rather than hyperglycaemia was implicated in their development (Fig. 2).

Another argument supporting the role of AGEs is the fact that they may accumulate in serum and tissues originating from diabetic patients [20, 34]. For instance, collagen extracted from the arterial walls of diabetic patients contained an increased amount of AGEs when compared with age-matched non-diabetic subjects [20]. The increase was highest in diabetic patients with ESRD, presumably because the kidney plays a major role in the elimination of AGEs [34]. In addition, large amounts of pyrraline (an AGE) accumulated in the glomeruli of patients with end-stage diabetic nephropathy [35]. *In vivo*, the prolonged administration of exogenous AGEs formed on albumin to normal animals resulted in considerable vascular dysfunction associated with unresponsiveness to vasodilatory agents. Normal rats injected for several months with AGEs developed renal and glomerular hypertrophy and mesangial sclerosis [33].

In the present study, we found that administration of AGEs to normal mice induced several changes characteristic of the early stages of diabetic nephropathy. The glomerular hypertrophy described in insulin-dependent diabetic patients [5] and experimental animals was reproduced by AGE injection. Within the kidney, we found that glomerular hypertrophy was not associated with a concomitant increase in total kidney size following AGE administration. This is consistent with our previous finding that total kidney and glomerular growth are independently regulated [26]. AGE-induced overexpression of laminin B1 and  $\alpha$ 1 IV collagen mRNAs was specific because the upregulation was prevented by the coadministration of aminoguanidine. Since there were no changes in  $\beta$ -actin or  $\alpha$ -smooth muscle actin, AGEs did not lead to a generalised upregulation of glomerular gene expression. Although interstitial collagen (type I collagen) has been reported in advanced diabetic glomerular lesions in humans, it was not part of the early response in

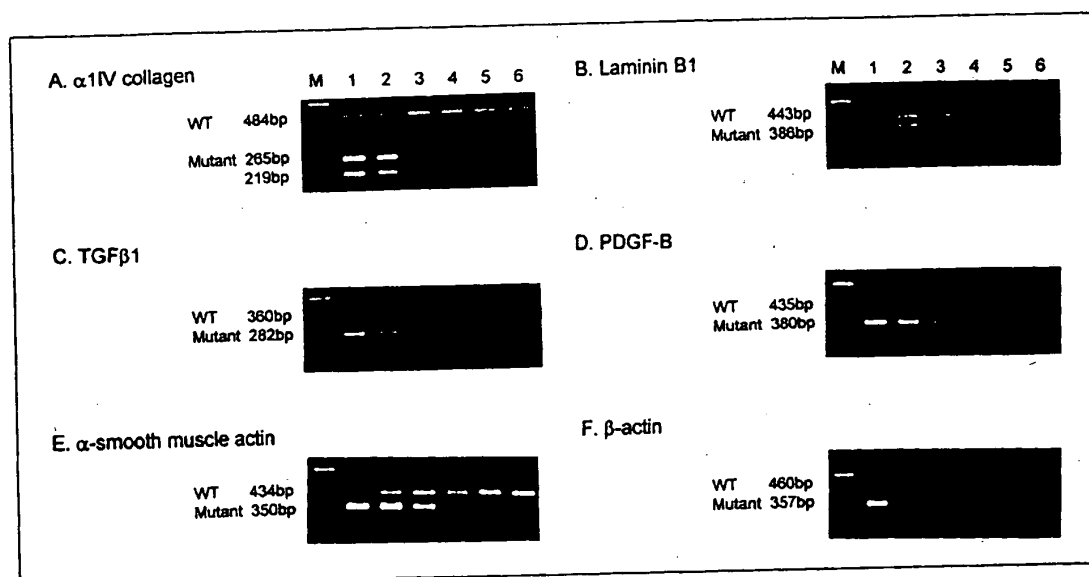


FIG. 2. mRNA expression as measured in isolated glomeruli from control, MSA, AGE-MSA and AGE-aminoguanidine-injected mice.

AGE-MSA mice [36]. We also found upregulation of TGF- $\beta$ , which has been reported to be elevated in the glomeruli of STZ diabetic rats [37].

## CONCLUSIONS

The results of the DCCT and other clinical trials provide strong evidence that close control of glucose to normal levels prevents the onset of nephropathy by nearly 50 % in early IDDM and delays the onset of nephropathy in patients with established retinopathy. There is considerable evidence that the deleterious effect of hyperglycaemia may be mediated by advanced glycosylation end-products. Our work has provided the first *in vivo* evidence that exogenous AGEs induce overexpression of glomerular ECM genes in normal mice. This data supports their role in mediating the development of diabetic nephropathy, independently of other metabolic or genetic factors.

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**ELSEVIER**  
**EDITORIAL ARTICLE****Prevention and slowing down the progression of the diabetic nephropathy through antihypertensive therapy.****Bretzel RG.**

Third Medical Department, University of Giessen, Germany.

Diabetic nephropathy is the major cause of illness and premature death in people with diabetes, largely through accompanying cardiovascular disease and end-stage renal failure. Diabetic patients are several times as prone to kidney disease as nondiabetic people and the accumulative risk of diabetic nephropathy in insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) is about 30%-50% after 25 years of disease. Diabetic nephropathy is a progressive disease that takes several years to develop, ending in chronic renal insufficiency. Proteinuria heralds the onset of diabetic nephropathy, and the worsening of proteinuria parallels progression of renal disease. The main risk factors for the frequency, severity and progression of diabetic nephropathy are the degree of hyperglycemia associated metabolic disturbances, hypertension, protein overload, cigarette smoking, as well as the duration of diabetes. Interventional strategies for primary, secondary, and tertiary prevention of diabetic nephropathy there include meticulous glycemic control, appropriate treatment of associated abnormalities, rigorous control of the blood pressure, reduction in dietary protein intake, in particular animal protein, and of fat intake, and stopping cigarette smoking. Randomized clinical trials indicate that antihypertensive therapy is beneficial in preventing and slowing down the progression of diabetic nephropathy. There is now increasing evidence that angiotensin-converting enzyme inhibitors and certain calcium antagonists produce a remarkable beneficial effect on diabetic nephropathy in terms of reducing proteinuria slowing the progression to diabetic renal failure. These drugs are attributed nephroprotective capacity beyond their blood pressure lowering capacity initial clinical trials with combinations have revealed even additive protective effects on end organs.

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## RENAL SYNDROMES IN DIABETES

Eli A. Friedman, MD

### GLOBAL IMPACT OF NEPHROPATHY DUE TO DIABETES

In 1995 according to registries of end-stage renal disease (ESRD) in the United States, Japan, and most nations in industrialized Europe, diabetes mellitus was the leading cause of renal failure worldwide. Other recognized etiologies of ESRD, including glomerulonephritis and hypertensive renal disease, are less prevalent in patients with new cases of ESRD. Mauer and Chavers<sup>102</sup> state, "Diabetes is the most important cause of ESRD in the Western world." According to the 1995 report of the United States Renal Data System (USRDS),<sup>160</sup> of 205,798 patients receiving either dialytic therapy or a kidney transplant in 1992, 54,586 had diabetes for a prevalence rate of 27.2%. Throughout 1992, of 54,586 new (incident) cases of ESRD, 19,790 (36.35%) were diagnosed as diabetes-related (Fig. 1).

Approximately 9% of Americans with diabetes have insulin-dependent diabetes mellitus (IDDM), whereas in contrast with as many as 50% of Norwegians with diabetes. Distinguishing IDDM from non-insulin-dependent diabetes mellitus (NIDDM) separates two disorders with dissimilar clinical courses. In a survey of race and gender among 232 diabetic patients in a group of 1450 (16%) undergoing maintenance hemodialysis at 14 centers in Brooklyn, the largest patient subset was a group of 87 black women, who comprised 37.5% of the total study population.<sup>93</sup> NIDDM was diagnosed without difficulty in 139 or 59.9% of diabetic patients undergoing hemodialysis, but diabetes type could not be determined in 24 (10.3%) patients.

Extensive overlap in signs and symptoms often blurs the distinction between types of diabetes. Nagai<sup>121</sup> found that of 551 patients diagnosed with diabetes before the age of 30 years, 337 (61.2%) had NIDDM. In Japanese patients with diabetes, diabetic retinopathy and nephropathy are as common in early

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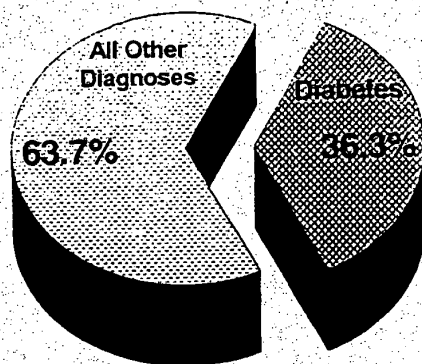


Figure 1. United States Renal Data System<sup>160</sup> compilation of newly started patients with ESRD during 1992 shows that more than one third had diabetes. Over the past 5 years, the relative rate of diabetic patients has been increasing progressively.

onset NIDDM as in IDDM. In their attempt to define the faulty limits of present classification systems for diabetes, Abourizk and Dunn<sup>1</sup> remarked that, "Clinicians treating diabetic patients encounter numerous insulin-taking diabetic subjects who clinically are neither IDDM nor NIDDM." These workers reviewed the records of 348 consecutive patients with diabetes and a mean age 53 years evaluated in Hartford and concluded that diabetes type could not be established in 35% of whites, 57% of blacks, and 59% of Hispanics. Until a new classification of diabetes is developed, recommendations pertaining to kidney transplantation in diabetes by diabetes type must be interpreted with caution. A combined pancreas-kidney transplant is applicable in IDDM but is probably not applicable in NIDDM.

#### FAMILIAL CLUSTERING

In some kindreds, a strong familial predisposition to kidney disease in diabetes is evident. Seaquist and co-workers<sup>143</sup> found a high risk of nephropathy in IDDM in families in which the first sibling in whom diabetes developed had a duration of diabetes of at least 10 years along with a duration of at least 7 years of urinary disease; only 2 of 12 IDDM sibling pairs free of diabetic nephropathy (17%) had urinary albumin excretion rates above 30  $\mu\text{g}/\text{min}$ . Obversely, in 12 of 29 (41%) sibling pairs in which one had ESRD, the other also had ESRD, whereas 12 others had albuminuria greater than microalbuminuria and only 17% were normal. In the aggregate, renal disease affected 82% of siblings whose probands had nephropathy. Borch-Johnsen and co-workers<sup>18</sup> also reported a higher prevalence of overt nephropathy in siblings of IDDM probands with nephropathy (33%) than in siblings of normoalbuminuric probands (10%,  $P < 0.04$ ). First-degree relatives of microalbuminuric patients with IDDM manifest more severe abnormalities of carbohydrate and lipid metabolism than do first-degree relatives of normoalbuminuric patients with IDDM.<sup>170</sup>

In two generations of Pima Indians with NIDDM, proteinuria occurred in 14% of offspring if neither parent had proteinuria, in 23% if at least one parent had proteinuria, and in 46% if both parents had diabetes and proteinuria.<sup>136</sup> A derivative population-based pedigree study in Arizona of Pima Indian offspring found that the prevalence of diabetes at ages 15 to 24 years and 25 to 34 years was 0% and 11%, respectively, if the diabetic parent did not have renal disease compared with 6% and 28%, respectively, if the diabetic parent did have renal disease. Offspring of two diabetic patients had corresponding rates of 10% and

17% if neither parent had renal disease compared with 30% and 50%, respectively, if one parent had renal disease.<sup>107</sup> Controlled for age, the odds of diabetes in an offspring was 2.5 times greater if a parent had renal disease.

Cardiovascular disease and cardiovascular death are more prevalent in parents of patients with IDDM who have nephropathy.<sup>46</sup> A positive family history of cardiovascular disease is much more frequent in diabetic patients with nephropathy or a prior cardiovascular event. Inferred from these findings is the conclusion that, in IDDM, a familial predisposition to cardiovascular disease increases the risk of nephropathy and the risk of cardiovascular disease in patients with nephropathy.

Characterization of the risk of hypertension and cardiovascular disease in relation to intermediate phenotypes, such as red blood cell sodium-lithium countertransport activity (Na/Li CT), suggests that increased activity of this membrane transport mechanism is associated with essential hypertension and some renal and cardiovascular complications.<sup>26</sup> Increased Na/Li CT is present in IDDM and NIDDM in patients with both microalbuminuria and clinical nephropathy.<sup>75, 83</sup> Parents of proteinuric patients with IDDM with high Na/Li CT also may have elevated values.<sup>163</sup> The prevalence of elevated Na/Li CT activity is 21.5%, 42.8%, and 51.7% in normoalbuminuric, microalbuminuric, and clinically proteinuric patients, respectively.<sup>91</sup> One study proposes an association between polymorphism of the insulin receptor gene and the development of overt proteinuria in patients with IDDM.<sup>42</sup> Thus, it seems that a familial predisposition to nephropathy is present in many, if not all, patients with diabetes who progress to ESRD.

#### DISTINGUISHING NEPHROPATHY IN IDDM FROM THAT IN NIDDM

As a generalization, the majority (70% to 95% depending on race) of persons with diabetes in Europe and the United States have NIDDM. Some population subsets, such as 100% full-blooded Native-Americans, have no IDDM despite a high prevalence of NIDDM. In both IDDM and NIDDM, ESRD is the end point of nodular and diffuse intercapillary glomerular sclerosis, following, in sequence, microalbuminuria, fixed proteinuria, a nephrotic syndrome, and azotemia.<sup>31, 42, 116</sup> After 20 to 30 years of IDDM, approximately 20% to 40% of patients manifest irreversibly failed kidneys.<sup>9</sup> Over the past 40 years, ESRD has developed in a decreasing proportion of patients with IDDM, reflecting enhanced blood pressure and blood glucose control. Whereas, previously, renal failure was thought to be relatively rare in NIDDM,<sup>61</sup> recent reports of defined populations followed longitudinally indicate an approximately equal risk of nephropathy in both major types of diabetes. In Rochester, New York, Humphrey and co-workers<sup>71</sup> found an equivalent rate of renal failure over 30 years in cohorts of 1832 patients with NIDDM and 136 patients with IDDM. Similarly, a series in Heidelberg, Germany, noted that after 20 years of diabetes, a serum creatinine level greater than 1.4 mg/dL was present in 59% of subjects with IDDM and 63% of subjects with NIDDM.<sup>66</sup> Thus, ESRD is not an unusual conclusion of diabetic nephropathy in NIDDM and may have an incidence approaching that in IDDM. Regardless of the incidence of ESRD in NIDDM, in the United States, Europe, and Japan, most patients with new cases of ESRD have NIDDM.

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## PATHOLOGY OF DIABETIC NEPHROPATHY

Autopsy analysis shows that vascular disease was the most common cause of death (38% to 48%) between 1958 and 1985 in Japanese patients with diabetes, who manifested a relatively constant rate of nephropathy and cerebrovascular diseases while coronary artery disease increased from 6.0% to 17%.<sup>60</sup> Early (several years after the onset of diabetes) morphologic changes in the diabetic kidney include thickening of the glomerular basement membrane (GBM),<sup>127</sup> as well as thickening of tubular basement membranes<sup>152</sup> and Bowman's capsule. GBM thickening is present within 1.5 to 2.5 years of the onset of IDDM. Increases in the volume of cellular and matrix components of the glomerular mesangium appear after 5 to 7 years of diabetes and within 2 to 5 years in a kidney from a nondiabetic donor transplanted into a diabetic recipient.<sup>103, 104</sup>

Changes in the GBM and mesangium are not highly correlated with one another; some patients have marked GBM thickening without much mesangial expansion, whereas others display the converse. Increased mesangium and GBM contain increased amounts of types IV and VI collagen, laminin, and fibronectin. With longer duration of diabetes or following the transplant of a normal kidney into a diabetic recipient, afferent and efferent arteriolar hyalinosis develops. This hyalinosis may progress to replacement of the smooth muscle cells in small vessels by a waxy, homogeneous, translucent-appearing material.

Mauer and colleagues<sup>106</sup> suggest that the key lesion of diabetic nephropathy leading to renal insufficiency in IDDM is expansion of the glomerular mesangium. Mesangial volume per glomerulus and glomerular volume, together, predict peripheral glomerular capillary filtration surface per glomerulus with great accuracy; filtration surface per glomerulus is highly correlated with glomerular filtration rate (GFR).<sup>130</sup>

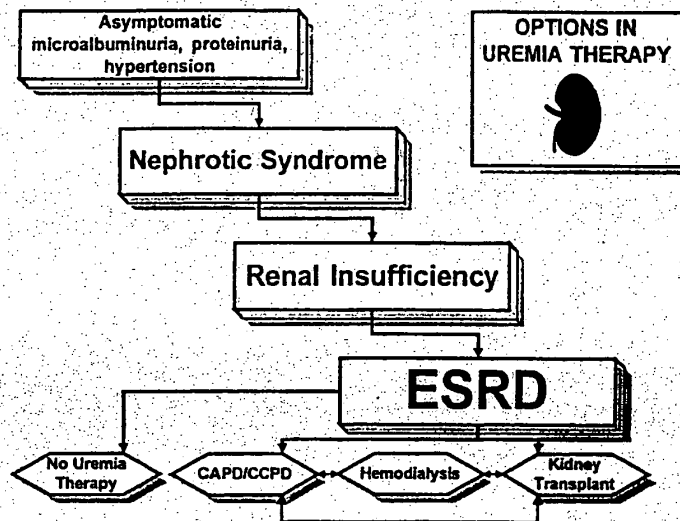
Mesangial expansion ultimately restricts glomerular capillary luminal volume, distorts glomerular capillary diameter and length relationships, and diminishes filtration surface.<sup>47</sup> All of the major manifestations of clinical diabetic nephropathy can be related to mesangial expansion and, necessarily, to distortions in glomerular capillary architecture. Diffuse glomerulosclerosis, the end effect of diffuse and generalized mesangial expansion, is the morphologic change exemplifying diabetic nephropathy; however, the histopathologic lesion most closely identified with diabetic nephropathy is nodular intercapillary glomerulosclerosis, a finding initially detected in a retrospective autopsy study of NIDDM by Kimmelstiel and Wilson.<sup>79</sup>

Nodular intercapillary glomerulosclerosis results from marked mesangial expansion forming large round fibrillar mesangial zones [on periodic acid-Schiff (PAS) stain], with palisading of mesangial nuclei around the periphery of the nodule and extreme compression of the associated glomerular capillaries. This generally focal and segmental lesion is the consequence of the dilatation of glomerular capillaries into microaneurysms,<sup>142</sup> an "exudative" lesion containing a variety of plasma proteins, especially immunoglobulins, complement, fibrinogen, and albumin.<sup>21, 110</sup> Other lesions also may be seen in the glomerular capillary subendothelial space (hyaline caps) and along the parietal surface of Bowman's capsule (capsular drops). Nodular changes may not be present despite advanced clinical diabetic nephropathy. Approximately 80% of patients with clinical nephropathy have few or no nodular lesions. With progressive decline in renal reserve, more and more glomeruli evince total sclerosis or have closure of glomerular capillary lumina in incompletely scarred glomeruli; however, no rigid relationship exists between the duration of IDDM and the severity of histopathologic changes.

Microalbuminuria signifies GBM and mesangial expansion<sup>31</sup>; higher levels of microalbuminuria indicate more advanced lesions and signal subsequent overt nephropathy. Normoalbuminuric patients with established lesions progress to microalbuminuria and overt nephropathy.<sup>32</sup> Albuminuria, therefore, is a marker of risk for nephropathy by virtue of indicating an underlying glomerular biochemical disturbance that promotes the accumulation of GBM and mesangial matrix material.<sup>34</sup>

### RENAL BIOPSY FOR DIAGNOSIS OF DIABETIC NEPHROPATHY

For the large majority of diabetic patients manifesting diabetic nephropathy, renal biopsy is unnecessary. In typical cases, comprising about four out of five patients, the diagnosis of diabetic nephropathy is securely based on clinical evidence. The sequence of syndromes, if followed sequentially as portrayed in Figure 2, is characteristic of the course in young individuals with IDDM. The development of overt proteinuria exceeding 0.5 g per 24 hours in a young patient after a decade or longer of IDDM, especially in the presence of diabetic retinopathy, signifies diabetic nephropathy with high reliability. The onset of overt proteinuria frequently is associated with hypertension and declining GFR. After the exclusion of other renal disorders by renal sonography, proteinuric patients with more than 10 years of IDDM, even in the absence of significant diabetic retinopathy, can be presumed to have diabetic nephropathy. Biopsy of the kidney, however, is appropriate in an atypical course (e.g., if proteinuria



**Figure 2.** Clinical renal syndromes in diabetic patients depicting the progression from nephropathy as a silent disorder characterized by microalbuminuria and hypertension through a nephrotic syndrome, azotemia, and ESRD. The choices available for management of ESRD are further divided by type of CAPD/CCPD, location of hemodialysis (i.e., facility or home), and source and type of organ transplant (i.e., kidney or kidney plus pancreas).

and rapid decline in renal function, clinical renal disease within the first 10 years of diabetes, or historical or laboratory findings support a different disease, such as a positive anti-DNA antibody titer indicative of lupus nephritis).

In NIDDM, especially in patients without retinopathy, the incidence of other renal diseases may exceed 25%, and renal biopsy may be a more important diagnostic tool.<sup>133</sup> Hematuria does not exclude diabetic nephropathy. It was observed in 26 of 154 patients with diabetes and was associated with IgA glomerulonephritis in 10 patients and membranous nephropathy in one patient.<sup>100</sup> When compared with diabetic patients without hematuria, those with hematuria had higher serum creatinine levels, worse retinopathy, and increased amounts of proteinuria. Hematuria in this series of Japanese diabetic patients indicated either a primary glomerulonephritis or an advanced stage of diabetic nephropathy. Renal biopsy is the best means to clarify the etiology of renal malfunction when the course is unusual.<sup>105</sup>

### RENAL INVOLVEMENT IN IDDM

Mogensen and colleagues<sup>116</sup> propose a five-stage sequence for renal involvement in IDDM.

#### Stage 1: Glomerular Hyperfiltration and Renomegaly

Recently diagnosed patients with IDDM have an increased inulin clearance first recognized in the 1930s and 40s.<sup>23</sup> Glomerular hyperfiltration has been confirmed by many workers.<sup>41</sup> GFRs of as much as 140% of normal values are seen,<sup>111</sup> and in some studies, elevation in the GFR is positively correlated with serum glucose concentration.<sup>168</sup> Most likely, both functional and structural abnormalities mediate glomerular hyperfiltration.<sup>27</sup> A closely linked relationship between hyperglycemia and hyperfiltration is evidenced by GFR decreases within 8 days of the initiation of insulin therapy.<sup>167</sup> Even with good or fair glucose control, however, the GFR remains above control levels in 25% to 40% of patients. In this subgroup of hyperfiltering patients, reductions in the GFR and clinical nephropathy eventually develop at a much greater rate than in control patients with diabetes with normal GFR. Microalbuminuria that is fully reversible on control of blood glucose also frequently is reported in this first stage.

#### Stage 2: Early Glomerular Lesions

Mild thickening of the GBM begins 18 to 24 months after the onset of IDDM and may be pronounced after 3.5 to 5 years.<sup>129</sup> GBM thickening, however, is present even in persons with diabetes who do not go on to have nephropathy.<sup>126</sup> The glomerular mesangial matrix also starts to expand after 2 to 3 years of disease<sup>128</sup> and increases out of proportion to the increase in glomerular volume. Exercise-induced microalbuminuria is the only clinical evidence of renal involvement during this stage, which may extend from 4 to 5 to 15 years following the diagnosis of diabetes.

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### Stage 3: Incipient Diabetic Nephropathy—The Microalbuminuric Stage

Hypertensive proteinuric patients with diabetes carry a poor prognosis.<sup>17</sup> Microalbuminuria, defined by a daily urinary albumin excretion rate of 20 to 200  $\mu\text{g}/\text{min}$ , predicts renal functional deterioration and a poor outcome.<sup>118</sup> Serial measurements of urinary albumin excretion are the distinct markers of the progression of diabetic nephropathy.<sup>115</sup> Patients with persistent microalbuminuria usually maintain their GFR; a subsequent fall in GFR is predicted by initial hyperfiltration.<sup>117</sup> With the development of proteinuria of greater than 300 mg/day, GFR in IDDM declines.<sup>50</sup> When microalbuminuria is present in IDDM, the degree of hypertension is not an added risk factor for a decline in GFR.<sup>124</sup>

Raised urinary albumin excretion is both a marker of and, perhaps, a determinant of future decline in renal function, although the nature of the initial glomerular permeability defect is unknown. Patients with a decline in GFR due to nephron closure show single-nephron hyperfiltration in remaining glomeruli. On the other hand, patients with microalbuminuria usually do not exhibit any decline in GFR, but some compensatory hyperfunction may still prevail in addition to the usual diabetic hyperfiltration.

Albuminuria is also associated with vascular damage in other organs.<sup>122, 119, 157</sup> Microalbuminuria is not detectable on dipstick urinalysis and, in its lower ranges, is not discovered on routine 24-hour urine protein screening. Quantitation of low concentrations of urinary albumin is performed by radioimmunoassay, nephelometric immunoassay, or enzyme-linked immunosorbent assay. A semiquantitative dipstick test also is available (Micro-Bumintest, Miles Laboratories, Elkhart, IN). Although it may initially be intermittent, microalbuminuria also may be exacerbated or caused by hypertension, hyperglycemia, exercise, urinary tract infections, hypervolemia, and protein loads. Inpatient day-to-day variability in urinary albumin excretions with coefficients of variation of approximately 45% has been reported.<sup>33</sup>

In 25% to 40% of individuals with IDDM, fixed microalbuminuria develops after 5 to 15 years of diabetes.<sup>134</sup> The majority of these patients have a progressive downhill course without intervention.<sup>113</sup> Approximately 40% of persons with IDDM have persistent hypertension ( $> 140/90$  mm Hg) along with microalbuminuria,<sup>16</sup> presumably related to the development of nephropathy, although renal biopsy confirmation is lacking.

### Stage 4: Clinical Nephropathy—Macroalbuminuria, Falling Glomerular Filtration Rate

In 20% to 40% of individuals with IDDM, proteinuria develops of greater than 200 to 300  $\mu\text{g}/\text{min}$  (300 to 500 mg/day). The incidence of macroalbuminuria peaks in patients who have had diabetes for 15 to 20 years. Without intervention, the GFR in macroalbuminuric patients with IDDM falls relentlessly at about 1 mL/min/month,<sup>112</sup> and urinary albumin excretion increases by about 2500  $\mu\text{g}/\text{min}/\text{year}$  with great interpatient variation. The nephrotic syndrome is common, and edema occurs at values of serum albumin much higher than that seen in other causes of nephrosis, perhaps, because of its conversion to glycated albumin which traverses capillary membranes more readily than normal albumin. The histologic presentation in macroalbuminuric patients with type 1 diabetes is that of well-established diffuse glomerulosclerosis; the typical

Kimmelstiel-Wilson lesions of nodular glomerulosclerosis are seen in only 50% of cases.<sup>57</sup>

### **Stage 5: End-Stage Renal Disease**

End-stage renal disease and its multiple complications and comorbid conditions occur after 20 to 30 years of diabetes in 30% to 40% of patients with IDDM. Uremic symptoms and signs are manifested at creatinine clearances that are higher than that in nondiabetic persons, and renal replacement therapy in suboptimally treated individuals is usually needed within 2 to 3 years of the onset of the nephrotic syndrome. The need for uremia therapy, however, may be postponed for months to years with regulation of blood pressure, dietary protein restriction, diuretics, and the use of erythropoietin in those patients with symptoms largely related to anemia.

### **RENAL INVOLVEMENT IN NIDDM**

Much less is known about the natural history of nephropathy in NIDDM than in IDDM, because of imprecision in the timing of the onset of NIDDM, which may be asymptomatic for years; 50% of patients with NIDDM are unaware of their disease, which is diagnosed either by random blood testing (pre-employment) or at discovery of a coincident illness.<sup>65</sup> Low estimates of the prevalence of clinical nephropathy or ESRD in NIDDM range from 2.5% to 10%.<sup>49</sup> Recent studies, however, indicate that nephropathy in NIDDM may occur at rates similar to in the IDDM population.<sup>66</sup> Such equivalence in attack rates has been reported in American Pima Indians,<sup>84</sup> blacks,<sup>36</sup> and Hispanics. The interval between the manifestation of NIDDM and the onset of ESRD ranges from 5 to 10 years; the older the patient at diagnosis of diabetes, the more rapid the progression to ESRD.<sup>123</sup>

Hyperfiltration is inconstantly documented in NIDDM. Fixed microalbuminuria is found in 20% to 37% of patients with newly diagnosed NIDDM.<sup>55</sup> The high prevalence of microalbuminuria in recently diagnosed NIDDM probably reflects a longer period of unrecognized disease or the presence of other factors known to cause microalbuminuria, such as hypertension, urinary tract infection, or nondiabetic glomerulopathy. The predictive value of microalbuminuria for subsequent ESRD in NIDDM is not well-established. The GFR may remain stable over many years of microalbuminuria or macroalbuminuria,<sup>54</sup> and it does not differ in normoalbuminuric and microalbuminuric patients with NIDDM.

### **RETARDING PROGRESSION OF DIABETIC NEPHROPATHY**

Three interventions slow the course of diabetic nephropathy.

#### **Blood Pressure Normalization**

Hypertension is a major contributor to the genesis and progression of diabetic nephropathy. Without question, the most important treatment compo-

nent in diabetic nephropathy is blood pressure reduction. As diastolic blood pressure rises in incipient diabetic nephropathy, microalbuminuria worsens and GFR falls. Systemic hypertension is deleterious because dilated afferent glomerular arterioles in the diabetic kidney transmit systemic blood pressure to glomeruli, further increasing glomerular capillary hypertension already present because of hyperfiltration with or without glomerular hypertrophy.<sup>70</sup> Blood pressure and the risk of nephropathy form a continuum, raising the questions of when to start treatment and what is the optimum target blood pressure. The Working Group on Hypertension in Diabetes<sup>169</sup> suggest that blood pressure be lowered to at least 140/90 mm Hg; however, it may be prudent to lower blood pressure to between 120 and 130/80 to 85 mm Hg.

Treatment of hypertension in the microalbuminuric stage slows its progression<sup>132</sup> even after macroalbuminuria ensues. With antihypertensive therapy, survival for more than 8 years in persons with IDDM and clinical or advanced nephropathy is improved from 48% to 87%.<sup>90</sup> In a study of hypertensive subjects with NIDDM for more than 10 years, 36% had impaired renal function defined as a GFR less than 80 mL/min/1.73 m<sup>2</sup> or a serum creatinine concentration greater than 1.4 mg/dL, and 75% had microalbuminuria or clinical proteinuria.<sup>29</sup> Treatment with captopril, an angiotensin-converting enzyme (ACE) inhibitor, administered for 18 months to 24 patients with NIDDM with proteinuria greater than 500 mg/day reduced proteinuria and prevented a decrease in GFR compared with the findings in 18 patients with NIDDM treated with conventional antihypertensive drugs.<sup>90</sup> Improvement in survival is related to the postponement of uremia and the reduction of cardiac disease and not to earlier use of dialysis or renal transplantation.

#### *Angiotensin-converting Enzyme Inhibitors: A Specific Role in Diabetic Nephropathy?*

Observations in experimental diabetes and other models of progressive renal disease suggest variable efficacy among antihypertensive agents in reducing proteinuria and slowing glomerular injury. In animal models of diabetic and other types of nephropathy, there is a selective effect of ACE inhibitors in decreasing glomerular pressure and single-nephron GFR and in retarding or abolishing glomerular injury.<sup>5</sup> In addition to their effect of decreasing systemic blood pressure, the beneficial hemodynamic effect of ACE inhibitors lies in the abolition of angiotensin II-mediated constriction of the efferent arteriole which contributes to glomerular hypertension. Mesangial cell proliferation and matrix production also may be inhibited, possibly by enhancing the cyclooxygenase pathway<sup>56</sup> with increased prostaglandin production. In hypertensive patients with incipient nephropathy, ACE inhibitors decrease the rate of fall of GFR by about 50% after 1 to 2 years of follow-up; however, control of blood pressure using other antihypertensive agents produces similar or better effects. Blood pressure control without the use of an ACE inhibitory drug results in an equivalent decline of creatinine clearance as has been reported in the best results using ACE inhibitors.<sup>135</sup>

In view of the efficacy of ACE inhibitors, the lack of side effects, and the good metabolic profile, the American Diabetes Association in its Clinical Practice Recommendations for 1995 states the following<sup>3</sup>:

Treatment of hypertensive IDDM patients who have microalbuminuria or clinical albuminuria with ACE inhibitors has been shown in clinical trials to delay progression from microalbuminuria to clinical albuminuria and to slow the decline in GFR in patients

with clinical albuminuria. Current data suggest that normotensive patients with albuminuria may also benefit from ACE inhibitors.

ACE inhibitors alone, however, frequently are inadequate to normalize hypertensive blood pressures in azotemic diabetic patients, especially in blacks. Furthermore, their side effects, especially cough, hyperkalemia, and an increase in azotemia, force drug withdrawal in approximately one fifth of predialysis diabetic patients. The next step in management is a combination of two or more antihypertensive drugs. Calcium channel blockers are excellent second-line therapy. Although diuretics may have adverse effects on glucose control and the lipid profile, they frequently are essential in attaining blood pressure control in hypoalbuminemic volume-overloaded patients with falling GFRs. Beta-blockers, central alpha-2-agonists (e.g., clonidine), or peripheral vasodilators (e.g., prazosin, hydralazine, minoxidil) also may be valuable adjuncts.

Adverse reactions to antihypertensive drug combinations limit their application. Diuretics may worsen diabetic control in a dose-related effect.<sup>25</sup> Small doses, such as furosemide, 40 to 80 mg/day, may not be diabetogenic nor increase blood glucose concentration. Potassium loss is important when GFR is near normal but can readily be restored by potassium supplementation or by the use of ACE inhibition. Small doses of diuretics may worsen hyperlipidemia, especially in hypoalbuminemic diabetic patients. Unselective beta-blockers may induce hypoglycemic unawareness, a dreadful imposition on the patient that is minimized by restricting treatment to cardioselective beta-blockers in the lowest effective doses. ACE inhibitors do not worsen glucose metabolism or lipid homeostasis; a beneficial effect has actually been observed in some studies. Insulin resistance may, in fact, decrease with ACE inhibition in NIDDM.<sup>158</sup> The use of minoxidil, the most powerful vasodilator, carries the risk of periodic hypotension, fluid retention, and prodigious hair growth over the face, hands, chest, and lower trunk. However, minoxidil does lower blood pressure in patients highly resistant to other hypertensive drugs. A succinct yet thorough up-to-date review of the pharmacology of antihypertensive drugs has been prepared by The Medical Letter.<sup>44</sup>

### Dietary Protein Restriction

Whether dietary protein restriction is beneficial in early diabetic nephropathy is unestablished; in advanced nephropathy, however, the value of a protein-restricted diet is supported by clinical trials. As is true in normal persons, dietary protein intake modulates renal hemodynamics in diabetic patients. The cumulative risk of diabetic nephropathy may be increased in individuals with IDDM who ingest a high-protein diet. Dietary protein raises single-nephron GFR and glomerular blood flow by producing afferent arteriolar vasodilatation in streptozotocin-induced diabetic rats.<sup>165</sup> Moderate and severe protein restriction early in the course of diabetes normalizes glomerular hypertension and single-nephron GFR in this model. A role for dietary protein restriction in IDDM has emerged, such as in nondiabetic renal disease, in which two to tenfold reductions in the rate of progression of renal failure have been reported.<sup>8</sup>

In a prospective, randomized, controlled study, 20 subjects with IDDM and clinical proteinuria (mean,  $3144 \pm 417$  mg/day) or renal impairment (iothalamate clearance,  $46 \pm 4.8$  mL/min/1.73 m<sup>2</sup>) fed a 0.6 g/kg/day protein diet over a mean follow-up of 34.7 months evinced a fourfold decrease in the rate of fall of GFR compared with that in 15 controls.<sup>171</sup> At the conclusion of the study, low

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protein-treated diabetic patients had a reduction of proteinuria by 6% (196 mg), whereas the controls had a 24% (1024 mg) increase. Proteinuria in nephrotic diabetic patients also may be reduced by dietary protein restriction.<sup>4</sup> Neither the time point in the course of diabetic nephropathy to start protein restriction nor the optimal level of protein intake are established.

In normoalbuminuric patients with IDDM, glomerular hyperfiltration is decreased by normalizing dietary protein intake,<sup>101</sup> a potential beneficial effect. Furthermore, one study suggests that microalbuminuria can be reduced by a low-protein diet.<sup>34</sup> Once nephropathy is advanced, as indicated by proteinuria in excess of 500 mg/day with or without azotemia, the rate of decline of GFR can be reduced by a low-protein diet.<sup>162</sup> In one study, patients were monitored as to their usual dietary intake of proteins and, thereafter, were started on a low-protein diet. A remarkable reduction in the rate of fall of the GFR was observed, although the response varied considerably. In a randomized parallel study, Zeller and co-workers<sup>171</sup> also documented the preservation of GFR in IDDM when nephropathy was advanced. The large multicenter Modification of Diet in Renal Disease (MDRD) study in the United States provided minimal support for advocating dietary protein restriction as a means of retarding nephropathy, but this trial included few diabetic patients.<sup>81</sup> Given the uncertainty over what constitutes proper protein intake in diabetes, we recommend a 0.6 to 0.8 g/kg/day protein diet in both IDDM and NIDDM once macroalbuminuria with or without a falling GFR is noted, providing that overall nutritional status is satisfactory.

### Glycemic Control

Microalbuminuria and late diabetic complications are associated with poor glycemic control. Longitudinal studies show that poor metabolic control, with glycosylated hemoglobin (HbA<sub>1c</sub>) levels of 7.5% or more, is common in patients with fixed microalbuminuria and increased GFRs. The interval between the onset of diabetes and the appearance of clinical proteinuria is shortened by poor glycemic control, and the risk for macroalbuminuria is four to five times greater in patients with poor control.<sup>125</sup> Strict glucose control in the 8-month Kroc study, the 2-year Steno study, the 3-year Dallas study, and the 4-year Oslo study all resulted in significant reductions in microalbuminuria in patients with IDDM. Based on the recently completed and massive Diabetes Control and Complications Trial (DCCT), the American Diabetes Association recommends the following<sup>2</sup>:

Setting individual patient glycemic targets should take into account the results of prospectively randomized clinical trials, most notably the Diabetes Control and Complications Trial (DCCT). This trial conclusively demonstrated that in patients with IDDM the risk of development or progression of retinopathy, nephropathy, and neuropathy is reduced 50% to 75% by intensive treatment regimens when compared with conventional treatment. These benefits were observed with an average hemoglobin A<sub>1c</sub> of 7.2% in intensively treated groups of patients compared with an average 9.0% in conventionally treated groups of patients.

In NIDDM, poor glucose control is associated with the development of clinical proteinuria and also correlates with the degree of structural renal damage and, possibly, the prevalence of uremia.<sup>22</sup> Data on the effect of tight glucose control on the prevention or regression of microvascular or macrovascular complications are almost entirely lacking.



## END-STAGE RENAL DISEASE IN DIABETES

Choices for the management of ESRD in patients with diabetes are as follows:

- Refusal of further treatment for uremia equates to passive suicide.
- Peritoneal dialysis
  - Machine-assisted intermittent peritoneal dialysis (IPD)
  - Continuous ambulatory peritoneal dialysis (CAPD)
  - Continuous cyclic peritoneal dialysis (CCPD)
- Hemodialysis
  - Facility hemodialysis
  - Home hemodialysis
- Renal transplantation
  - Cadaver donor kidney
  - Living related-donor kidney
  - Living unrelated-donor kidney (emotionally related donor)
- Pancreas plus kidney transplantation
  - IDDM
  - ?NIDDM

Diabetic patients with ESRD are managed similarly to nondiabetic patients with two exceptions: (1) simultaneous pancreas and kidney transplantation is a diabetes-specific therapy and (2) no treatment, meaning passive suicide, is the choice more often selected for and by diabetic individuals than by nondiabetic individuals. Although the objective of uremia therapy is to permit an informed patient to select from a menu of available regimens, realities of program resources usually channel the diabetic patient with ESRD to the treatment preferred by the supervising nephrologist. As a consequence, CAPD may be the first choice in Toronto, home hemodialysis in Seattle, and a renal transplant in Minneapolis. No prospective controlled trials of dialytic therapy of any type versus kidney transplantation have been reported. For these and other unexplored reasons, diabetic patients with ESRD are less likely than nondiabetic patients to be selected for a kidney transplant, and, therefore, a greater proportion are treated by hemodialysis (Fig. 3).

Confusion over the type of diabetes is common in the evaluation of diabetic patients with ESRD. Confounding the distinction of the diabetes type is the realization that, in Sweden, as many as 14% of cases originally diagnosed as NIDDM progressed to IDDM, whereas 10% of newly diagnosed diabetic individuals could not be classified.<sup>15</sup> Diabetes in the United States is predominantly NIDDM, fewer than 10% of diabetic Americans are insulinopenic, C-peptide negative persons who have IDDM. ESRD in diabetic persons reflects the demographics of diabetes, *per se*,<sup>172</sup> in that the incidence<sup>69</sup> is higher in women, blacks,<sup>153</sup> Hispanics,<sup>64</sup> and Native-Americans,<sup>172</sup> and the peak incidence of ESRD occurs from the fifth to the seventh decade. Inferred from these relative attack rates is the reality that blacks over the age of 65 years face a seven times greater risk of diabetes-related renal failure than do whites. Therefore, in the United States, it is not surprising that ESRD associated with diabetes is mainly a disease of poor, elderly blacks.<sup>35</sup>

Vasculopathic complications of diabetes are at least as severe in NIDDM as in IDDM.<sup>102, 109</sup> In fact, recognition of the high prevalence of proteinuria and azotemia in carefully observed subjects with NIDDM contradicts the view that NIDDM infrequently induces nephropathy. Although there are differences between patients with IDDM and NIDDM in genetic predisposition<sup>146</sup> and racial expression, other aspects of the two disorders, particularly manifestations of

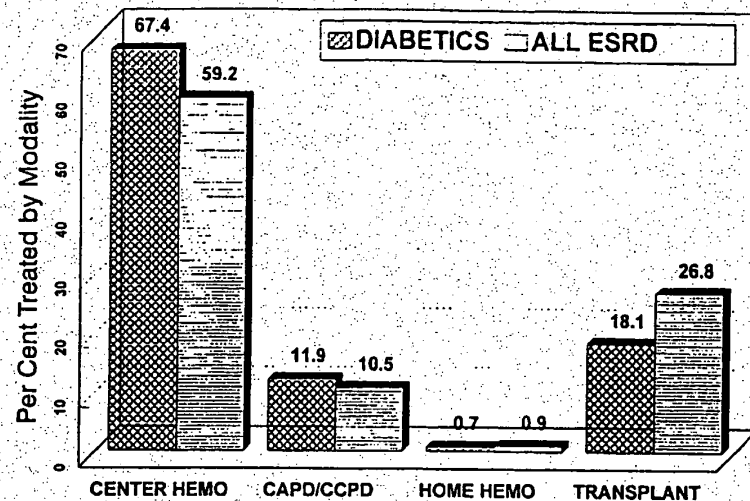


Figure 3. In practice, according to the United States Renal Data System in 1992, diabetic ESRD patients are less likely to receive a kidney transplant, meaning that a greater proportion are treated with hemodialysis or peritoneal dialysis.

nephropathy are remarkably similar. Lack of precision in diabetes classification provokes confusing terms, such as "insulin requiring" to explain treatment with insulin in persons thought to have resistant NIDDM. In fact, the currently used criteria are unable to classify as many as one half of diabetic persons as having IDDM or NIDDM.<sup>1,148</sup> Consequently, reports in the literature of the outcome of therapy for ESRD by diabetes type are few and imprecise.

### COMORBID RISK FACTORS

Management of diabetes in a person with progressive renal insufficiency is more difficult than in an age- and gender-matched nondiabetic person. The toll of coincident extrarenal disease, especially blindness, limb amputations, and cardiac disease, limits or preempts rehabilitation. For example, the creation of a hemodialysis vascular access in a nondiabetic patient is minor surgery but in a diabetic patient, risks major morbidity from infection or deranged glucose regulation. As a group, diabetic patients manifesting ESRD have a higher death rate because of cardiac decompensation, stroke, sepsis, and pulmonary disease than do nondiabetic patients with ESRD (Figs. 4 and 5). Depression caused by multiple complications during dialytic therapy prompts a substantially higher rate of withdrawal from therapy (suicide) in diabetic than in nondiabetic patients with ESRD. The major comorbid concerns in the management of diabetic patients with ESRD include the following:

- Decreasing visual acuity, retinopathy, glaucoma, cataracts
- Coronary artery disease: congestive heart failure, cardiomyopathy
- Dizziness, transient ischemic attacks, cerebrovascular disease

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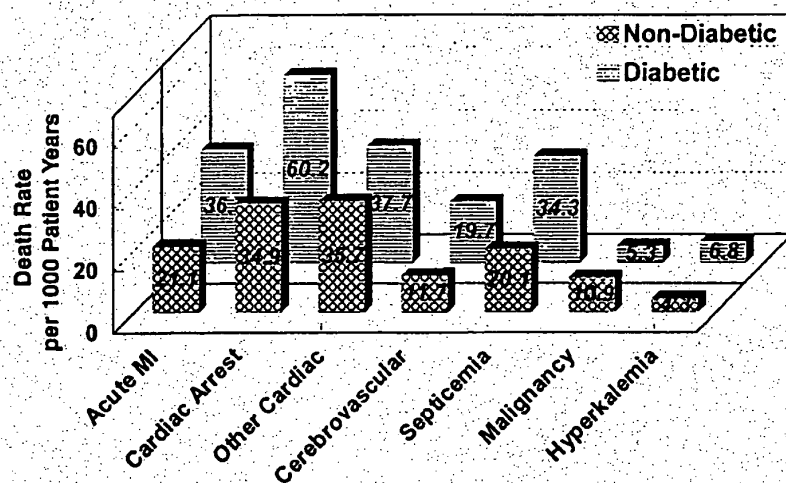


Figure 4. Comorbid extrarenal disease is responsible for the majority of deaths in diabetic and nondiabetic patients with ESRD treated with both peritoneal and hemodialysis. The dominance of cardiac disorders is evident from these data detailing death rates reported to the United States Renal Data System<sup>100</sup> for 1991 and 1992.

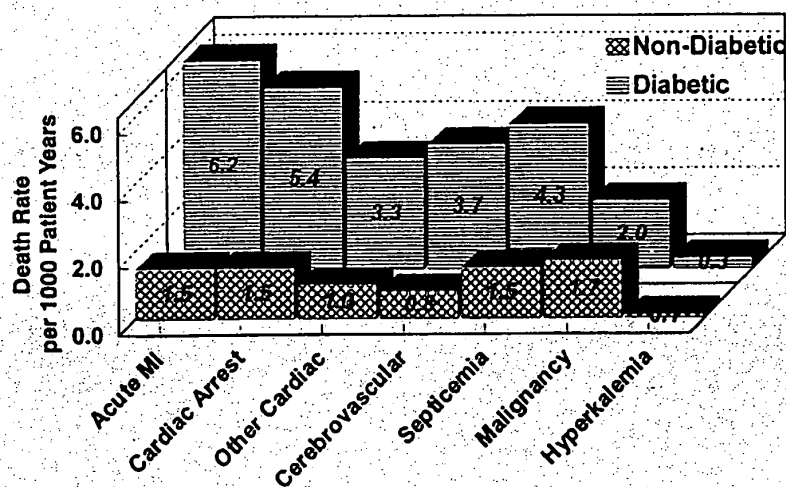


Figure 5. Comorbid extrarenal disease accounts for most deaths in diabetic and nondiabetic renal transplant recipients. The dominance of cardiac disorders is evident from these data detailing death rates reported to the United States Renal Data System<sup>100</sup> for 1991 and 1992.

Restless legs, leg cramps, peripheral vascular disease, limb amputation  
 Motor neuropathy, sensory neuropathy  
 Autonomic dysfunction: diarrhea, dysfunction, orthostatic hypotension  
 Myopathy  
 Depression, sometimes suicidal

### Eye Disease

Diabetic retinopathy exceeds heart and lower limb disease as the major concern in overall patient care. When careful medical observation has been used, approximately 100% of diabetic individuals with ESRD have undergone laser treatment with or without vitrectomy for retinopathy. More than 24,000 new cases of blindness in the United States are attributed to diabetic retinopathy each year.<sup>166</sup> In Seattle, of newly evaluated diabetic patients with ESRD, about 97% have significant retinopathy,<sup>14</sup> and 25% to 30% are blind or have severe vision loss.<sup>59</sup> Macular edema, glaucoma, cataracts, and corneal disease also must be considered in diabetic patients facing blindness.<sup>74</sup> In Minneapolis between 1966 and 1971, 44% of newly treated patients with IDDM and undergoing hemodialysis sustained progressive visual loss culminating in blindness, but the rate of lost vision fell to 20% between 1972 and 1975 and to 4% thereafter.<sup>145</sup> Aggressive treatment of all aspects of the pathophysiology of diabetes, emphasizing blood pressure control plus well-timed ophthalmologic intervention, is credited for the preservation of sight. A lesson well learned is that laser surgery of the retina, that is, focal or panretinal laser photocoagulation, halts previously inexorable proliferative retinopathy. As D'Amico<sup>37</sup> proposes, periodic assessment by a retinal surgeon including properly timed panretinal photocoagulation and vitrectomy together with intensive treatment of blood glucose regulation and control of hypertension remarkably preserves sight.

Retinopathy progressing to visual loss heralds a poor prognosis for either long-term survival or rehabilitation. As illustrated by the series of Diglas and co-workers,<sup>40</sup> of 157 patients followed up for a mean of 5.1 years after laser treatment, one-quarter (24.2%) of the study population died, 18.3% had a myocardial infarction, 14.7% had a limb amputated, and 8.3% had uremia, but only 7.3% became blind. Blindness in diabetic patients with ESRD is avoidable. This assertion is sustained by multiple reports exemplified by the study of Wantanabe and colleagues.<sup>164</sup> In their series of 268 Japanese patients with diabetes and undergoing hemodialysis, of whom 50% survived 60 months, there was stable visual acuity in 364 of 418 eyes (87.1%), whereas 20 of 418 eyes improved and only 34 of 418 eyes (8.1%) deteriorated. The author's experience in Brooklyn validates the theory that integrated laser and vitreous surgery applied throughout the stages of nephrotic syndrome and azotemia stops the loss of vision.<sup>11</sup>

### Heart Disease

Significant ischemic heart disease, defined as 70% or greater occlusion of at least one coronary artery,<sup>19</sup> threatens the survival of diabetic patients; as many as 70% with this finding sustain a major cardiovascular event within 5 years.<sup>72, 98</sup> Even with minimal degrees of coronary artery stenosis (25% to 75%), 50% of diabetic subjects sustain a severe cardiovascular event in 5 years. Because it is believed that the correction of coronary occlusion reduces cardiovascular mor-

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bidity and mortality after renal transplantation, pretransplant cardiac screening is now routine in diabetic patients with ESRD. Benefit from such screening is inferred from the report by Manske and co-workers<sup>47</sup> who noted that whereas 10 of 13 patients with coronary obstruction experienced a cardiac event within 8 months of follow-up when treated with calcium channel blockers and aspirin, only 2 of 13 sustained an event after a revascularization procedure.

Thallium scintigraphy is a noninvasive effective method for screening diabetic patients with ESRD. In a prospective study of renal transplant recipients, Le and co-workers<sup>46</sup> noted that only 2 of 42 high-risk patients with a negative thallium study pretransplant died of cardiac death, whereas 13 of 53 patients with an abnormal scan pretransplant died. Within the diabetic subset of subjects, cardiac mortality was 0% with a negative thallium scan but was 29% in those with a reversible defect.

A decade earlier, however, Morrow and co-workers<sup>130</sup> found thallium scanning less valuable because of an inability to attain maximal exercise in diabetic subjects. Modifying thallium testing using dipyridamole may have an advantage in improving the accuracy of the detection of coronary occlusion. In a review by Bia and Matthiesson<sup>12</sup> of a total of 196 diabetic patients in three reports between 1989 and 1993, fewer than 5% of diabetic and nondiabetic patients with ESRD with a negative dipyridamole thallium screening test experienced a cardiac event over 1 to 2 years of follow-up regardless of whether renal transplantation was performed. Dobutamine echocardiography has been proposed to screen sedentary diabetic patients for coronary artery disease. In a prospective study of 753 diabetic and nondiabetic patients, dipyridamole-thallium 201 imaging was a powerful predictor of 82 subsequent cardiac events in subjects with coronary disease and a low exercise tolerance.<sup>88</sup> Some transplant groups advocate direct visualization of the coronary circulation by angiography as the most reliable method of identifying diabetic patients with ESRD at risk for death following kidney transplantation.<sup>96</sup>

All diabetic uremic patients should undergo periodic cardiac evaluation. When under consideration for organ transplant surgery, a stress thallium scintigraphic study should be attempted in all diabetic subjects who can exercise to a maximum. Should intensive exercise not be possible, a dipyridamole thallium study should be performed. A positive test is an indication for coronary catheterization to guide in subsequent angioplasty or coronary artery bypass surgery.

### Limb Amputation

At least 15% of diabetic patients will have a foot ulcer at some time, and, of these, 15% to 20% undergo lower limb amputation.<sup>87</sup> Foot ulcers in patients with diabetes are the end product of coincident peripheral vascular disease and autonomic and somatosensory neuropathy.<sup>68</sup> Although it may seem obvious, a Swedish prospective study documents the decidedly lower cost of curing diabetic foot ulcers using a multidisciplinary team approach when compared with the expense of limb amputation.<sup>6</sup> Initiating a program to educate the patient about potential limb disease together with frequent examination of the feet is a highly effective means of preventing limb loss.<sup>24</sup> Defensive medicine,<sup>147</sup> a prime example of no-cost care, consists of daily washing, drying, and examination of the nails, soles, and interdigital creases of the feet, and wearing comfortable nonconstricting shoes with socks or stockings during the day and heel booties when confined to bed. Monthly visits to a podiatrist for nail and callous care complete the ideal program of preventive foot care. In appreciation of the often

severe visual loss that accompanies renal insufficiency, diabetic patients are instructed not to cut their own toenails because of the real risk of self-inflicted injury; a small cut risks infection, retarded healing, and eventual limb amputation. The author concurs with Bridges and Deitch<sup>31</sup> who view diabetic foot infections as a "failure by the patient and his management team to understand and correct the multifactorial conditions that predisposed the patient to the infection."<sup>135</sup> Osteomyelitis should be suspected in all diabetic foot ulcers that are slow to heal.<sup>45</sup> Contemporary vascular surgery diagnostic techniques, including limb examination by Doppler sonography and plethysmography to document specific mechanisms of limb hypoperfusion, point to the consideration of vascular bypass surgery or limb-preserving minimal amputation. For those patients considered too high-risk for conventional bypass surgery, percutaneous transluminal angioplasty may successfully restore foot perfusion.<sup>45</sup>

### Neurologic Complications

Azotemic diabetic patients have an increased rate of cerebrovascular accidents (strokes), transient ischemic attacks, and altered intellect because of decreased cerebral perfusion resulting from macrovasculopathy of cerebral arteries. During the management of ESRD, deaths caused by cerebrovascular disease are about twice as common in patients with diabetes. Diabetes compounds the risk of stroke and transient ischemic attack, not only by promoting cerebral atherogenesis but also by aggravating other risk factors, including hypertension, heart disease, and hyperlipidemia.<sup>76</sup> Treatment options for stroke in diabetic patients with renal insufficiency require individualization but should incorporate risk factor modification, especially normalization of hypertensive blood pressure, and may include aspirin, platelet antiaggregants, anticoagulation, or, in a well-defined subgroup, carotid endarterectomy.<sup>13</sup>

Sensory and motor neuropathy are common in long-standing diabetes. It may be impossible to discern the precise cause of an impaired ability to walk or even stand without assistance in a diabetic patient who is catabolic because of renal insufficiency. Uremic and diabetic neuropathy are indistinguishable by usual light microscopic techniques. Should motor neuropathy progress during adequate hemodialysis, a trial of CAPD is reasonable.

Autonomic neuropathy, expressed as gastropathy, cystopathy, and orthostatic hypotension, is a frequently overlooked, highly prevalent disorder affecting the quality of life in the diabetic patient with ESRD. Diabetic cystopathy, although common, is frequently unrecognized and confused with worsening diabetic nephropathy and is sometimes misinterpreted as allograft rejection in diabetic kidney transplant recipients. In a study of 22 diabetic patients in whom renal failure developed including 14 men and 8 women with a mean age of 38 years, an air cystogram detected cystopathy in eight patients (36%) manifested as detrusor paralysis in one patient, severe malfunction in five patients (24%), and mild impairment in one patient. Gastroparesis affects one quarter to one half of azotemic diabetic persons when they are initially evaluated for renal disease.<sup>32</sup>

Impaired gastric emptying (gastroparesis) affects about one half of all diabetic patients<sup>69</sup> and is present on initial evaluation for renal disease in the majority of azotemic diabetic persons.<sup>32</sup> The establishment of careful metabolic regulation is hindered by delayed gastric emptying, which is more common than rapid emptying. Confirmation of gastroparesis is made by a radionuclide gastric motility study, which, if positive, prompts treatment with metoclopra-

mide (preferably in liquid form), cisapride, or erythromycin. The consensus of gastroenterologists is that metoclopramide is the best first-line therapy for gastroparesis with cisapride a fair alternative in resistant cases.<sup>43</sup> Other expressions of gastrointestinal autonomic neuropathy, such as obstipation and explosive nighttime diarrhea, often coexist with gastroparesis.<sup>10</sup> Obstipation responds to daily doses of cascara, whereas diarrhea is treated with psyllium seed dietary supplements one to three times daily plus loperamide<sup>44</sup> in repetitive 2 mg doses to a total dose of 18 mg daily.

Complicating the rehabilitation of the patient with diabetes by therapy for ESRD is an incompletely understood restrictive arthropathy of the hand, finger, shoulder, and hip joints.<sup>7</sup> The diabetes-induced joint disease is difficult to distinguish from other arthropathies that affect the long-term dialysis patient.

### CHOICES IN THERAPY FOR END-STAGE RENAL DISEASE

Depending on age, the severity of comorbid disorders, available local resources, and patient preference, the uremic diabetic patient may be managed according to different protocols (Table 1). Diabetic patients with ESRD select the no further treatment option, equivalent to passive suicide, more frequently than do nondiabetic patients.<sup>108</sup> Such a decision is understandable for blind, hemiparetic, bed-restricted limb amputees for whom the quality of life has been reduced to what is interpreted as unsatisfactory. On the other hand, attention to the total patient may restore a high quality of life that was unforeseen at the time of ESRD evaluation<sup>80</sup> (Table 1).

### Maintenance Hemodialysis

For most of patients in the United States, that is, for more than 80% of diabetic persons in whom ESRD develops, maintenance hemodialysis is the only renal replacement regimen that is used. Approximately 12% of diabetic persons with ESRD are treated by peritoneal dialysis, whereas the remaining 8% receive a kidney transplant. Maintenance hemodialysis requires the establishment of a vascular access to the circulation. Creation of what has become the standard access—an internal arteriovenous fistula in the wrist—is often more difficult in a diabetic than in a nondiabetic person because of advanced systemic atherosclerosis. For many diabetic patients with peripheral vascular calcification with or without atherosclerosis, creation of an access for hemodialysis necessitates the use of synthetic prosthetic vascular grafts. The typical hemodialysis regimen requires three weekly treatments lasting 4 to 5 hours each, during which extracorporeal blood flow must be maintained at 300 to 500 mL/min.

Motivated patients trained to perform self-hemodialysis at home attain the longest survival and best rehabilitation afforded by any dialytic therapy for diabetic ESRD. When hemodialysis is performed at a facility, however, diabetic patients fare less well, receiving significantly less dialysis than nondiabetic patients, in part because of hypotension and reduced access blood flow.<sup>30</sup> Maintenance hemodialysis does not restore vigor to diabetic patients, as documented by Lowder and co-workers<sup>93</sup> in 1986, who reported that of 232 diabetic patients undergoing maintenance hemodialysis, only 7 were employed, whereas 64.9% were unable to conduct routine daily activities without assistance. Approximately 50% of diabetic patients in the United States who are started on maintenance hemodialysis die within 2 years of the first dialysis treatment.

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Table 1. OPTIONS IN THERAPY FOR ESRD IN DIABETIC PATIENTS

Variable	Peritoneal Dialysis	Hemodialysis	Kidney Transplant
Extensive extrarenal disease	No limitation	No limitation except for hypotension	Excluded in cardiovascular insufficiency
Geriatric patients	No limitation	No limitation	Arbitrary exclusion as determined by program
Complete rehabilitation	Rare, if ever	Very few individuals	Common, so long as graft functions
Death rate	Much higher than for nondiabetic patients	Much higher than for nondiabetic patients	About the same as for nondiabetic patients
First-year survival	About 75%	About 75%	>90%
Morbidity during first year	About 15 days in hospital	About 12 days in hospital	Weeks to months hospitalized
Survival to second decade	Almost never	Fewer than 5%	About 1 in 5
Progression of complications	Usual and unremitting; hyperglycemia and hyperlipidemia	Usual and unremitting; may benefit from metabolic control	Interdicted by functioning pancreas plus kidney; partially ameliorated by correction of azotemia
Special advantage	Can be self-performed; avoids swings in solute and intravascular volume level	Can be self-performed; efficient extraction of solute and water in hours	Cures uremia; freedom to travel
Disadvantage	Peritonitis; hyperinsulinemia; hyperglycemia, hyperlipidemia; long hours of treatment; more days hospitalized than with either hemodialysis or transplant	Blood access a hazard for clotting, hemorrhage, and infection; cyclical hypotension, weakness, aluminum toxicity, amyloidosis	Cosmetic disfigurement, hypertension, personal expense for cytotoxic drugs; induced malignancy; HIV transmission
Patient acceptance	Variable, usual compliance with passive tolerance for regimen	Variable, often noncompliant with dietary, metabolic, or antihypertensive component of regimen	Enthusiastic during periods of good renal allograft function; exalted when pancreas proffers euglycemia
Relative cost	Most expensive over long run	Less expensive than kidney transplant in first year, subsequent years more expensive	Pancreas plus kidney engraftment most expensive uremia therapy for diabetic; after first year, kidney transplant alone is lowest cost option



### Peritoneal Dialysis

In the United States, peritoneal dialysis sustains about 12% of diabetic patients with ESRD. CAPD offers the advantages of freedom from a machine, performance at home, rapid training, minimal cardiovascular stress, and avoidance of heparin.<sup>89</sup> To permit CAPD, an intraperitoneal catheter is implanted 1 or more days before CAPD is begun. Even blind diabetic patients can learn to perform CAPD at home within 10 to 30 days. Typically, CAPD requires the exchange of 2 to 3 L of sterile dialysate containing insulin, antibiotics, and other drugs three to five times daily. Mechanical cycling of dialysate, termed *continuous cyclic peritoneal dialysis* (CCPD) or *automatic peritoneal dialysis* (APD), can be performed during sleep. CAPD and CCPD pose the constant risk of peritonitis and a gradual decrease in peritoneal surface area. Some clinicians characterize CAPD as a "first-choice treatment" for diabetic patients with ESRD.<sup>90</sup> A less enthusiastic judgment of the worth of CAPD in diabetic patients was made by Rubin and Hsu<sup>141</sup> in a largely black diabetic population in Jackson, Mississippi. Only 34% of patients remained on CAPD after 2 years, and, at 3 years, only 18% continued on CAPD. According to the USRDS, the survival of diabetic patients with ESRD who are treated by CAPD is significantly less than that of similar patients undergoing hemodialysis. The decision to select CAPD, therefore, must be based on the individual patient after weighing its benefits, including the freedom from a machine and electric outlets and the ease of travel, against the disadvantages of unremitting attention to fluid exchange, the constant risk of peritonitis, and disappearing exchange surface. As concluded in a *Lancet* editorial,<sup>137</sup> "Until the frequency of peritonitis is greatly reduced, most patients can expect to spend only a few years on CAPD before requiring a different form of treatment, usually haemodialysis."

### Kidney Transplantation

As predicted by Sutherland and co-workers,<sup>155</sup> following a renal transplant, patient survival at 1 and 2 years is equivalent in diabetic and nondiabetic recipients,<sup>151</sup> though graft survival remains marginally lower in diabetic persons. As illustrated by a single-center retrospective review of all kidney transplants performed between 1987 and 1993, at best, there is no significant difference in actuarial 5-year patient or kidney graft survival between diabetic and nondiabetic recipients overall or when analyzed by donor source. Furthermore, no difference in mean serum creatinine levels at 5 years was noted between diabetic and nondiabetic recipients.<sup>144</sup> Statistical superiority in survival after a renal transplant, when compared with dialytic therapy, does not tell the whole story as rehabilitation is incomparably better. The author holds that the enhanced quality of life affected prompts the selection of a kidney transplant as the preferred regimen presented to newly evaluated diabetic persons with ESRD who are aged less than 60 years. More than half of diabetic kidney transplant recipients in most series live for at least 3 years. Many survivors return to occupational, school, and home responsibilities.

### Pancreas Plus Kidney Transplantation

Positioning the option of a combined pancreas and kidney transplant for the diabetic patient with ESRD is difficult. Although still regarded as investiga-

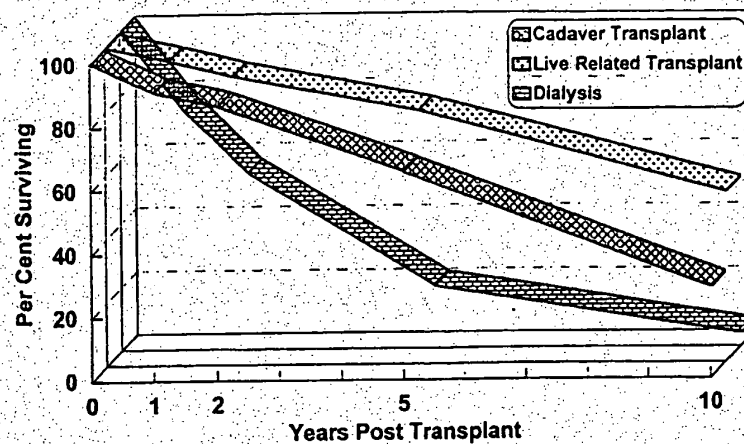
tional by some<sup>140</sup> and, even when successful, applicable to no more than the 9% subset of uremic diabetic patients who have IDDM, pancreatic transplantation is growing in acceptability and technical success.<sup>141</sup> In one remarkable series, survival 1 year post renal transplant in 995 diabetic kidney recipients who also received a pancreas transplant, renal allograft survival was a remarkable 84%.<sup>28</sup> Worldwide results in simultaneous kidney-pancreas transplants show that more than 90% of recipients live 1 year, more than 80% have functioning kidney grafts, and more than 70% no longer require insulin.<sup>154</sup> Although combining pancreas and kidney transplants does not raise perioperative mortality, perioperative morbidity is greatly increased, mainly because of mechanical and inflammatory problems in diverting pancreatic exocrine secretions into the urinary system.

A great expectation for pancreatic transplantation is interdiction of diabetic microvascular and macrovascular extrarenal complications. Several reports of kidney biopsies performed in patients given sequential kidney and pancreas allografts indicate that a functioning pancreas does impede the progression or recurrence of diabetic nephropathy. Likewise, the course of diabetic neuropathy following combined pancreas and kidney transplantation suggests stabilization and, in some patients, improvement in diabetic motor neuropathy.<sup>78</sup> Unfortunately, although pancreas transplantation in patients with extensive extrarenal disease has been reported to improve autonomic dysfunction,<sup>67</sup> in short-term observation it neither arrests nor reverses diabetic retinopathy, diabetic cardiomyopathy, or extensive peripheral vascular disease.<sup>138, 139</sup> Nevertheless, a functioning pancreas transplant does free patients with IDDM from the dreaded daily sentence of balancing diet, exercise, and insulin dosage,<sup>77</sup> and their quality of life is remarkably improved.<sup>48</sup> In 1995, the consensus of clinical nephrologists was that the patient with IDDM who has ESRD should consider a simultaneous kidney and pancreas transplant as, at least, a temporary cure for inexorable disease.<sup>156</sup>

## SURVIVAL DURING TREATMENT OF END-STAGE RENAL DISEASE

All reported comparisons (retrospective and prospective) of the fate of diabetic patients treated for ESRD by different modalities lack balanced treatment groups in terms of equalities in age, race, diabetes type, severity of complications, and the degree of metabolic control (Fig. 6). Prospective studies of renal transplantation compared with peritoneal or hemodialysis do not overcome limitations imposed by patient and physician refusal to allow random assignment to one treatment over another. As a generalization, younger patients with fewer complications are assigned to renal transplantation, whereas residual older sicker patients are treated by dialysis. Combined kidney-pancreas transplants are restricted to patients with IDDM who are younger than 50 years.

Diabetes adds a severe restriction to survival after the onset of ESRD, imparting a threefold rise in the risk of dying when compared with either chronic glomerulonephritis or polycystic kidney disease. In England, diabetic and nondiabetic patients starting CAPD or hemodialysis in seven large renal units between 1983 and 1985 were monitored prospectively over 4 years. Of 610 new patients (median age, 52 years, range, 3 to 80 years) beginning CAPD and 329 patients (median age, 48 years; range, 5 to 77 years) starting hemodialysis, patient survival estimates at 4 years were 74% for hemodialysis and 62% for CAPD.<sup>58</sup> Survival during CAPD and maintenance hemodialysis is lower in the



**Figure 6.** Relative survival rates during a decade in diabetic patients with ESRD treated with renal transplantation or dialytic therapy tabulated in the 1995 report of the United States Renal Data System.<sup>100</sup> Note both the dismal survival of dialysis patients and the marked superiority of living-donor over cadaver-donor renal transplant recipients.

United States than in Europe. An explanation for the better survival of diabetic patients undergoing dialysis in Europe is not evident, although the growing tendency in the United States to reuse dialyzer and shorten treatment hours has been incriminated as promoting fatal underdialysis.<sup>53</sup> Currently, substantiation of the superiority of one treatment for ESRD over another is lacking, whether for the total population of patients with ESRD or for the subset with diabetic nephropathy (see Table 1).

## REHABILITATION

Inferences extracted from the study of rehabilitation in the diabetic patient with ESRD are that patients fare best when participating in their treatment regimen, and that a functioning renal transplant permits markedly superior rehabilitation than that attained by either peritoneal dialysis or hemodialysis. Unfortunately, bias in assignment to a specific treatment may have prejudiced the favorable view of kidney transplants to the extent that statistical corrections (Cox proportional hazards technique) cannot compensate for group differences. Studies in which the mean age of transplant patients is a decade younger than that of the CAPD or hemodialysis groups are likely to discern better functional status in the younger group. Another variable affecting the magnitude of rehabilitation attained in diabetic and nondiabetic patients with ESRD is the progressive increase in age of newly treated patients. In the United States, for example, patients aged more than 69 years, who comprised 27% of all dialysis patients in 1979, increased by 450% between 1974 and 1981 and will make up 60% of all dialysis patients by the year 2010. An aging population with ESRD has a declining rate of employment and increasingly prevalent comorbid complications.

In an attempt to judge rehabilitation in patients undergoing maintenance hemodialysis, Gutman and co-workers<sup>63</sup> measured functional assessment in 2481 patients irrespective of location or type of dialysis.<sup>63</sup> Diabetic patients achieved

very poor rehabilitation. Only 23% of diabetic patients (versus 60% of nondiabetic patients) were capable of physical activity beyond caring for themselves. Lowder and co-workers discerned the same low level of rehabilitation. More recent confirmation of this finding has been provided by Ifudu and co-workers<sup>73</sup> who documented pervasive failed rehabilitation in a multicenter study of diabetic and nondiabetic<sup>73</sup> and elderly inner-city<sup>72</sup> hemodialysis patients. Although life-prolonging, neither maintenance hemodialysis nor peritoneal dialysis provides satisfactory rehabilitation for uremic diabetic individuals. By contrast, at least one half of diabetic renal transplant recipients can return to job, home, or school activities, achievements that score as satisfactory rehabilitation. Of course, the selection bias in determining whether healthier patients are given organ transplants may account for much of the superior outcome of renal transplantation over dialytic therapy in diabetic patients.

### ALTERNATIVE STRATEGIES TO MODIFY THE COURSE OF NEPHROPATHY

The importance of the burden of increased amounts of advanced glycosylated end products (AGEs) that are known to accumulate in diabetic patients with ESRD has yet to be established. Uremia in diabetes is associated with both a high serum level of AGEs and accelerated macrovasculopathy and microvasculopathy. The renal clearance of AGE peptides is  $0.72 \pm 0.23$  mL/min for normal subjects and  $0.61 \pm 0.2$  mL for patients with diabetes with normal glomerular filtration ( $P = \text{NS}$ ).<sup>161</sup> Diabetic uremic patients accumulate AGEs in toxic amounts that are not decreased to normal by hemodialysis or peritoneal dialysis<sup>131</sup> but fall sharply to within the normal range within 8 hours of restoration of half-normal glomerular filtration by renal transplantation.<sup>95</sup> The author hypothesizes that one reason for the better outcome after a kidney transplant is the prompt excretion of AGEs by the new kidney.

Diabetes modifies the rheologic properties of blood, increasing its viscosity and making erythrocytes less flexible. Pentoxifylline, a drug that improves peripheral and central circulation in diabetes,<sup>159</sup> has been reported sporadically to induce remarkable benefit in diabetic nephropathy in Spain and Russia<sup>149, 150</sup> in IDDM and NIDDM. In a prospective double-blind trial in Mexico, 41 nephropathic patients with IDDM and 45 patients with NIDDM were randomized to receive either pentoxifylline or placebo.<sup>62</sup> Pentoxifylline reduced microalbuminuria to zero in both IDDM and NIDDM, whereas placebo induced no change. Similarly, clinical proteinuria decreased with pentoxifylline but not placebo in both diabetes types. Further attention to this fresh approach to the management of diabetic microvasculopathy is warranted. The potential applications of aldose reductase inhibitors and aminoguanidine to block adverse metabolic reactions leading to tissue and organ damage are discussed elsewhere in this issue.

### COMORBID INDEX FOR DIABETIC PATIENTS

To facilitate inpatient and outpatient comparisons over the course of treatment of ESRD, the staff at the author's institution inventories the type and severity of common comorbid problems. Numeric ranking of this inventory constitutes a comorbid index, listed as follows:

1. Persistent angina or myocardial infarction
2. Other cardiovascular problems, hypertension, congestive heart failure, cardiomyopathy

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3. Respiratory disease
4. Autonomic neuropathy (gastroparesis, obstipation, diarrhea, cystopathy, orthostatic hypotension)
5. Neurologic problems, cerebrovascular accident, or stroke residual
6. Musculoskeletal disorders, including all varieties of renal bone disease
7. Infections including AIDS but excluding vascular access site or peritonitis
8. Hepatitis, hepatic insufficiency, enzymatic pancreatic insufficiency
9. Hematologic problems other than anemia
10. Spinal abnormalities, lower back problems, or arthritis
11. Vision impairment (minor to severe—decreased acuity to blindness)
12. Limb amputation (minor to severe—finger to lower extremity)
13. Mental or emotional illness (neurosis, depression, psychosis)

To obtain a numerical comorbidity index for an individual patient, rate each variable from 0 to 3 (0 = absent, 1 = mild or of minor importance to patient's life, 2 = moderate, 3 = severe). By proportional hazard analysis, the relative significance of each variable can be isolated from the other 12. Comparison between treatments (hemodialysis versus CAPD versus renal transplantation versus combined kidney and pancreas transplantation) demands that patient subsets be equivalent in severity of illness before application of the treatment modality under study.

The complexities of fusing multiple specialties to collaborate in the patient's interest are best handled by identification of a team capable of monitoring the patient's course while having the skills to apply needed interventional therapies.

This management team for uremic diabetic patients with ESRD should include the following:

- Nephrologist
- Vascular surgeon
- Transplant surgeon
- Diabetatologist
- Nutritionist
- Podiatrist
- Ophthalmologist
- Nurse educator
- Social worker
- Clergy advisor
- Social support system member

Without such cooperation and collaboration, the patient may be torn between conflicting opinions and local "turf" disputes. To expedite management of the myriad microvascular and macrovascular complications which accompany kidney failure in diabetic nephropathy, an inventory of comorbid risk factors should be taken. Subsequent selection of therapy for ESRD in a diabetic individual whose kidneys are failing requires a team approach and appreciation of the patient's family, social, and economic circumstances. Home hemodialysis, for example, is unworkable for a blind diabetic individual who lives alone. Deciding on a kidney transplant requires knowledge of the patient's family structure, including its willingness to participate by donating a kidney. Without premeditation, the diabetic patient with ESRD is subjected to repetitive, inconclusive studies instead of implementation of urgently required treatment (such as pan-retinal photocoagulation or arterial bypass surgery).

The strategy may involve no treatment when life extension is unacceptable.

A blind hemiparetic diabetic patient experiencing daily angina and nocturnal diarrhea who is scheduled for bilateral lower limb amputation may choose death despite his or her family's plea that he or she start maintenance dialysis. Because azotemic diabetic patients typically are depressed, however, a rational decision to die must be distinguished from temporary despair over a current setback. Despondent diabetic patients, on occasion, respond to visits by rehabilitated dialysis patients or transplant recipients by reversing their decision to die. It is unwise to coerce the acceptance of dialysis or a kidney transplant when life has minimal (or even negative) value. Diabetic patients forced into uremia therapy by family or the health care team are often noncompliant to dietary and drug regimens, thereby expressing behavior which culminates in passive suicide.

A functioning kidney transplant provides the uremic diabetic patient a greater survival with greater rehabilitation than does either CAPD or maintenance hemodialysis. This progress in therapy reflects multiple small advances in understanding the pathogenesis of extrarenal microvasculopathy and macrovasculopathy in an inexorable disease coupled with safer immunosuppression.

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## Leukocyte analysis using monoclonal antibodies in human glomerulonephritis

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Leukocyte analysis using monoclonal antibodies in human glomerulonephritis. The leukocyte subpopulations were analyzed within both the glomeruli and the interstitium in renal biopsies from 145 patients with various forms of glomerulonephritis. Cells were identified by monoclonal antibodies to leukocyte cell-surface antigens and immunoperoxidase labelling. Leukocytes, as defined by a monoclonal antibody to the leukocyte common antigen (PHM1), were present in normal, human renal tissue in both glomeruli ( $2.8 \pm 0.6$  cells/glom. cross section) and interstitium ( $102 \pm 18$  cells/mm<sup>2</sup>). Monocytes constituted the predominant infiltrating cell type in normal glomeruli ( $1.3 \pm 0.2$ ) and T cells were rarely found ( $0.3$ ; range 0 to 0.8), whereas both monocytes ( $34 \pm 10$ /mm<sup>2</sup>) and T lymphocytes ( $33 \pm 14$ /mm<sup>2</sup>) were found in the normal interstitium. In the non-proliferative forms of glomerulonephritis there was no significant increase in the number of glomerular inflammatory cells when compared with normal glomeruli. However, significantly increased numbers of T lymphocytes were seen in the interstitium of biopsies with minor non-specific changes ( $67 \pm 15$ /mm<sup>2</sup>), membranous nephropathy ( $134 \pm 30$ /mm<sup>2</sup>), focal glomerulosclerosis ( $207 \pm 53$ /mm<sup>2</sup>), and diabetic nephropathy ( $198 \pm 81$ /mm<sup>2</sup>). In the proliferative forms of glomerulonephritis only crescentic GN and post-infectious GN demonstrated significantly increased glomerular monocytes and granulocytes. There was no significant increase in the number of glomerular T cells when compared with normal glomeruli. However, there was a significant increase in the number of interstitial T lymphocytes in all forms of proliferative glomerulonephritis when compared with the normal interstitial cell population. In particular, this was seen in post-infectious GN ( $183 \pm 49$ /mm<sup>2</sup>), IgA nephropathy ( $283 \pm 59$ /mm<sup>2</sup>), diffuse mesangial proliferative lupus nephritis ( $215 \pm 64$ /mm<sup>2</sup>), crescentic GN ( $508 \pm 101$ /mm<sup>2</sup>), membranoproliferative GN ( $481 \pm 127$ /mm<sup>2</sup>) and focal proliferative GN ( $289 \pm 92$ /mm<sup>2</sup>). There was no significant difference in the OKT4:OKT8 ratio compared with that in the normal interstitium. There was a weak negative correlation only between glomerular leukocyte accumulation and renal function (as measured by creatinine clearance) ( $r = 0.27$ ,  $P < 0.01$ ), whereas there was a strong correlation between interstitial leukocyte accumulation and decline in creatinine clearance ( $r = -0.61$ ;  $P < 0.001$ ). This study demonstrates and characterizes the interstitial leukocytic infiltrate, predominantly T lymphocyte, in human glomerulonephritis and shows a strong correlation with impairment of renal function.

The pathogenesis of glomerulonephritis is primarily due to immune mechanisms. Although the role of humoral immunity has been well established [1, 2] the contribution of cellular immune mechanisms involving monocytes and T-lymphocytes is less clear [3].

Cellular immune mechanisms can only be said to play an unequivocal part in the pathogenesis of a particular disease when that disease can be induced in a syngeneic animal by the transfer of sensitized lymphocytes, but not by the transfer of serum alone. Although this has recently been accomplished in animal models of both nephrotoxic nephritis and immune complex-mediated glomerulonephritis [4, 5], this approach is clearly not applicable to the investigation of human glomerulonephritis. More indirect approaches have therefore been devised to assess the cellular arm of the immune response in patients with glomerulonephritis [6]. One such approach in human glomerulonephritis is to attempt to identify the components of a cell mediated immune response *in situ*. Examination of the glomeruli using monoclonal antibodies has demonstrated monocytes, with no significant increase in lymphocytes, in crescentic GN and post-infectious GN. There was no significant increase in glomerular mononuclear leukocytes in any other form of glomerulonephritis.

In contrast, interstitial mononuclear hypercellularity has been a recognized feature of several forms of glomerulonephritis [7]. However, until monoclonal antibody technology became available it was not possible to characterize these mononuclear cells. The aims of this study were, therefore, to characterize the leukocytic participants *in situ*, both within the glomerulus (substantially extending our previous observations) and in the interstitium, using a panel of monoclonal antibodies which are highly specific and sensitive markers of leukocyte subpopulations.

A significant interstitial, mononuclear leukocytic infiltrate was found in all forms of GN except minimal change nephropathy, and the effect of this infiltrate on renal function was also evaluated.

### Methods

#### Tissue

Diagnostic renal biopsies from 145 patients with glomerulonephritis were studied over a period of 2½ years. The biopsies were essentially sequential, although some patients who underwent renal biopsy did not have sufficient material and other biopsies were omitted because of technical difficulties in processing. Thirteen control normal sections were obtained from cadaver nephrectomies unused for transplantation and kidneys removed for localized tumors. All tissue specimens were routinely processed for light microscopy, immunofluorescence and

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Table 1. Monoclonal and antibody specificities

Monoclonal antibody	Specificity	Reference
PHM 1	Common leucocyte antigen (Not expressed on renal interstitial dendritic cells)	[9,10]
OKM 1	Monocytes, granulocytes, subset T cells	[11]
FMC 32	Monocytes and renal inter- stitial dendritic cells	[12,10]
FMC 10	Granulocytes	[13]
9.6 (Iy 3)	Total T lymphocytes	[14]
OKT 4	Helper/inducer T lymphocytes	[15]
OKT 8	Suppressor/cytotoxic T lymphocytes	[16]
PHM 14, B-1	B lymphocytes	[17,18]
Leu-7	NK cells	[19]

electron microscopy. In addition, a portion of each renal biopsy was processed for immunoperoxidase staining with monoclonal antibodies. These specimens were fixed with freshly prepared periodate-lysine-paraformaldehyde (PLP) for two hours at 4°C [8], washed in several changes of PBS containing 7% sucrose, snap frozen and stored at -80°C. Cryostat sections (6 µm) were prepared on the day of staining and adhesion to microscope slides was achieved by prior gelatin coating of the slides.

#### Monoclonal antibodies

A panel of monoclonal antibodies to leukocyte cell surface antigens was used for analysis of renal leukocytes. Table 1 lists the characteristics of these monoclonal antibodies.

#### Tissue localization

A sensitive four layer peroxidase-antiperoxidase (PAP) technique was used. Briefly, as previously described [8], sections were incubated sequentially with monoclonal antibody; rabbit anti-mouse immunoglobulin (RAM) 1:400 for 15 minutes, swine anti-rabbit immunoglobulin (SAR) (Dako) 1:40 for 15 minutes, and peroxidase-antiperoxidase (rabbit) complexes (PAP) (Dako) 1:100 for 30 minutes, followed by 3', 5', diaminobenzidine (DAB) and hydrogen peroxide for three to five minutes. Sections were counterstained with hematoxylin, then dehydrated, mounted and examined using a Leitz Dialux microscope (Leitz Inc., Heerbrugg, Switzerland). Endogenous peroxidase activity was blocked by adding 0.3% sodium azide to the DAB solution. Specificity of labelling was shown by lack of staining following substitution of PBS for the primary monoclonal antibody. Positive staining of interstitial leukocytes provided a positive internal control in those sections where there were no identifiable intraglomerular leukocytes. This was particularly important in the assessment of intraglomerular lymphocytes.

#### Quantitation

**Glomerular leukocytes.** The number of glomeruli available for counting varied from three per section to 20 per section. For each biopsy the number of labelled cells was counted in each glomerulus and expressed as the number of cells per glomerular cross section. The total leukocyte numbers were identified using the McAb to the common leukocyte antigen (PHM1); however, in 12 biopsies within the post-infectious and crescentic

groups, the total glomerular leukocyte count was derived from the sum of the subpopulations of leukocytes.

**Interstitial leukocytes.** Cells in the interstitium were counted using an eye piece graticule to identify ten microscopic fields, each 0.02 mm<sup>2</sup>, and hence a total area of 0.2 mm<sup>2</sup> was counted. This approximated to an area equivalent to 25 glomerular cross sections. The numbers were then expressed as cells per mm<sup>2</sup>. The sections were counted in a sequence of adjacent fields, and no adjustment of field was made except to avoid glomeruli and large vessels. Again, the total cell numbers were identified using PHM1, the common-leukocyte antigen marker, which was shown to provide dense labelling and reliable staining. All other cell counts were expressed as a percentage of this total cell number. Because of the patchy nature of the infiltrate this occasionally led to minor discrepancies between the total cell numbers as defined by PHM1 and the total obtained from the sum of the cells labelled by individual monoclonal antibodies. For each histological group, numbers were expressed as the mean cells per square millimeter ± standard error of the mean (SEM).

#### Renal function

Serum creatinine was determined and endogenous 24 hour creatinine clearance was used to measure the glomerular filtration rate. This functional assessment was carried out at the time of the biopsy. Because of the circumstances which usually surround kidney procurement, renal function and urinary parameters were incomplete for many normal kidneys and hence no valid comparison of creatinine clearance and urinary indices between normal and diseased states could be made.

#### Statistical analysis

The data were analyzed on a Digital Vax Computer using programs from the SPSS Batch System. Generally, non-parametric statistics were employed because of the relatively small numbers in each group. In particular, the Mann-Whitney U, Wilcoxon rank sum test was applied to compare absolute numbers of cells between groups, and the Kolmogorov-Smirnov 2-sample test was used to compare proportions of cells between groups. Correlations were determined using Spearman's test. When very small numbers were seen, means were expressed with ranges rather than SEM, and where large numbers were involved, Student's *t*-test was applied.

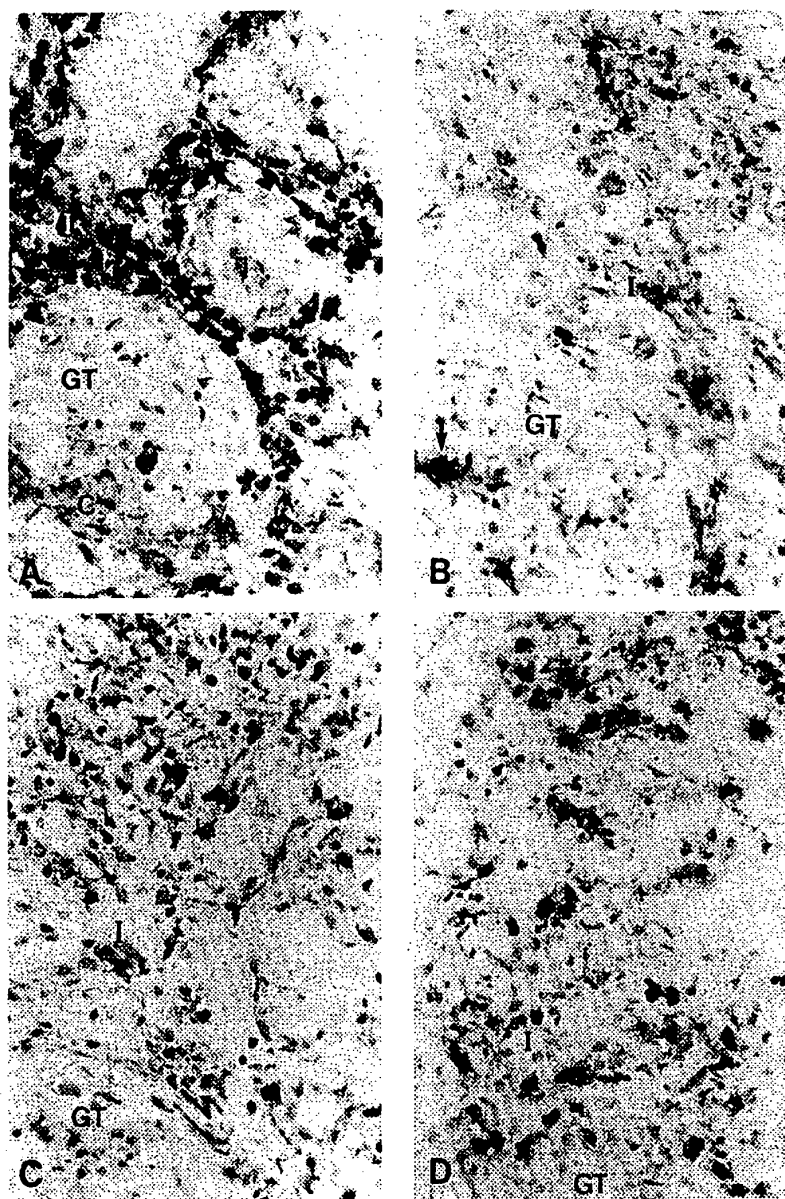
#### Results

Figure 1 illustrates the morphology and staining of sections of a renal biopsy from a patient with crescentic glomerulonephritis. There is a prominent periglomerular infiltrate composed mainly of T lymphocytes. Both OKT4<sup>+</sup> and OKT8<sup>+</sup> lymphocytes are demonstrated. There are monocytes but no T lymphocytes within the glomerulus, but both monocytes and T lymphocytes are present in the interstitium.

#### Glomerular leukocyte infiltration (Table 2)

**Normal tissue.** The mean intraglomerular leukocyte count, as defined by the leukocyte common antigen (PHM1), was 2.8 ± 0.6 (SEM) cells per glomerular cross section. This total comprised monocytes, granulocytes and an occasional T lymphocyte. B cells and NK cells were only rarely identified. Cells labelled by the monoclonal antibody OKM1 (monocytes and





**Fig. 1.** Immunoperoxidase staining of a renal biopsy from a patient with crescentic glomerulonephritis. A. There is a prominent periglomerular mononuclear cell infiltrate which is positively labelled with the monoclonal antibody to the common leukocyte antigen (PHM1). There are positively staining leukocytes also within the glomerular tuft and the glomerular crescent. B. OKM1 labels monocytes within the glomerular tuft (arrow) and a small number of cells within the interstitium. C. OKT4 labels cells within the renal interstitium but none are demonstrated within the glomerular tuft. D. OKT8 labels a similar number of cells within the renal interstitium as OKT4. Although the glomerular tuft is only partly shown, it can be seen from Table 3 that overall, very few lymphocytes were demonstrated within glomeruli. Abbreviations are: GT, glomerular tuft; C, crescent; I, renal interstitium.

polymorphs) accounted for  $3.3 \pm 1.0$  cells per glomerular cross section, providing confirmation of the above figures.

**Non-proliferative glomerulonephritis.** There was no significant difference between total intraglomerular leukocyte counts when the disease states in this category were compared to normal. The main, identified cell type had the monocyte/macrophage phenotype and an occasional granulocyte was seen. T

cells, B cells and NK cells were rarely identified within the glomerulus. Comparison of individual cell types between normal glomeruli and non-proliferative glomerulonephritides demonstrated significant decreases in intraglomerular monocytes in those biopsies showing mild non-specific abnormalities, membranous nephropathy and focal glomerulosclerosis. In the minimal lesion group there was a significant decrease in cells



Table 2. Intraglomerular leukocytes

	Number of patients	Total leukocytes	Monocytes	Granulocytes	OKM1 +ve cells	T Lymphocytes	B Lymphocytes
Normal	13	2.8 ± 0.6 <sup>a</sup>	1.3 ± 0.2	1.0 ± 0.3	3.3 ± 1.0	0.3(0-0.8) <sup>b</sup>	0.04(0-0.4)
<b>Non-Proliferative GN</b>							
Non-spec.	19	2.2 ± 0.7	0.6 ± 0.1 <sup>d</sup>	0.4 ± 0.1 <sup>d</sup>	1.3 ± 0.3 <sup>d</sup>	0.3(0-1.5)	0.08(0-0.7)
Min. les.	4	2.9 ± 1.7	NA	0 <sup>d</sup>	0.4 ± 0.3 <sup>d</sup>	1.3(0.6-2.0)	0.3(0-1.0)
Membr.	13	2.8 ± 1.1	0.4 ± 0.2 <sup>e</sup>	0.4 ± 0.4 <sup>d</sup>	1.6 ± 0.8 <sup>d</sup>	0.2(0-1.0)	0
FGS	13	2.0 ± 0.6	0.6 ± 0.3 <sup>d</sup>	0.6 ± 0.3	2.3 ± 0.7	0.1(0-0.5)	0.1(0-0.3)
Diab.	9	2.1 ± 0.6	1.2 ± 0.4	0.3 ± 0.2	0.9 ± 0.5 <sup>d</sup>	0.6(0-1.0)	0.1(0-0.2)
<b>Proliferative GN</b>							
PINF	8	31.7 ± 8.8 <sup>f</sup>	18.7 ± 7.2 <sup>e</sup>	17.1 ± 7.5 <sup>e</sup>	25.5 ± 5.9 <sup>e</sup>	1.0(0-2.0)	NA <sup>c</sup>
Idio.	6	2.5 ± 1.2	NA	3.8(0-7.5)	6.8 ± 0.8	0.1(0-0.2)	NA
IgA	18	3.8 ± 0.7	0.7 ± 0.3	0.8 ± 0.3	3.3 ± 0.7	0.7(0-2.3)	0.04(0-0.2)
SLE	13	3.3 ± 0.6	1.5 ± 0.5	0.4 ± 0.2	2.3 ± 0.9	0.1(0-0.5)	0.1(0-0.5)
Cresc.	14	13.1 ± 6.0 <sup>f</sup>	7.5 ± 2.1 <sup>e</sup>	5.9 ± 4.4	5.0 ± 1.6	0.6(0-2.3)	0
MPGN	8	12.0 ± 4.9	4.4 ± 1.7	2.9 ± 1.8	10.8 ± 4.5	0.2(0-0.6)	1.4(0-3.0)
Focal	10	3.1 ± 1.5	0.9 ± 0.4	1.4 ± 1.3	2.9 ± 1.0	1.1(0-3.5)	0.5(0-2.5)
Interst. neph.	10	2.7 ± 0.8	0.9 ± 0.6	1.0 ± 0.6	2.6 ± 1.0	0.5(0-1.3)	0.02(0-0.1)

Abbreviations are: Non-spec., minor non-specific glomerular abnormalitis; Min. les., minimal lesion GN; Membr., membranous GN; FGS, focal glomerulosclerosis; Diab., diabetic nephropathy; PINF, postinfectious GN; Idio., idiopathic mesangial proliferative GN; IgA, diffuse mesangial proliferative GN due to mesangial IgA deposition; SLE, diffuse proliferative GN due to systemic lupus erythematosus; Cresc., crescentic GN; MPGN, membranoproliferative GN; Focal, focal proliferative GN; Interst. neph., interstitial nephropathy.

<sup>a</sup> Expressed as cells per glomerular cross-section (mean ± SEM)

<sup>b</sup> Where numbers were very small, ranges have been used

<sup>c</sup> No data available

<sup>d</sup>  $P < 0.05$

<sup>e</sup>  $P < 0.01$

<sup>f</sup>  $P < 0.001$

Table 3. Intraglomerular leukocytes according to immunoglobulin deposition

	Total leukocytes	P	Monocytes	P	Granulocytes	P	T Lymphocytes	P
IgG -ve	4.1 ± 0.8 <sup>a</sup>	<0.05	1.7 ± 0.5	<0.05	1.1 ± 0.3	NS	0.5 ± 0.1	NS
+ve	9.6 ± 2.6		4.5 ± 1.3		3.8 ± 1.7		0.4 ± 0.1	
IgM -ve	10.4 ± 2.3	<0.001	5.5 ± 1.5	<0.01	4.0 ± 1.5	<0.05	0.5 ± 0.1	NS
+ve	2.5 ± 0.3		0.9 ± 0.2		0.5 ± 0.1		0.4 ± 0.1	
IgA -ve	7.4 ± 1.8	NS	3.3 ± 0.9	NS	3.0 ± 1.3	NS	0.5 ± 0.1	NS
+ve	5.6 ± 1.7		2.8 ± 1.3		1.6 ± 0.9		0.4 ± 0.1	
C <sub>3</sub> -ve	4.2 ± 0.9	NS	1.3 ± 0.4	<0.05	1.1 ± 0.5	NS	0.7 ± 0.3	NS
+ve	7.1 ± 1.5		3.4 ± 0.9		2.7 ± 1.0		0.4 ± 0.1	
Fibrinogen -ve	4.3 ± 0.8	<0.05	2.1 ± 0.7	NS	0.9 ± 0.3	<0.05	0.4 ± 0.1	NS
+ve	9.4 ± 2.5		4.2 ± 1.3		4.3 ± 1.8		0.5 ± 0.1	

NS, Not significant ( $P > 0.05$ )

<sup>a</sup> Expressed as cells per glomerular cross section (mean ± SEM)

labelled by OKM1. There was a significant reduction in OKM1 labelled cells in diabetic nephropathy  $0.9 \pm 0.5$  compared with normals  $3.3 \pm 1.0$  ( $P < 0.05$ ).

**Proliferative glomerulonephritis.** A significant increase in the total number of infiltrating leukocytes (labelled by PHM1) was seen in the glomeruli of patients with post-infectious glomerulonephritis ( $31.7 \pm 8.8$ ;  $P < 0.001$ ) and crescentic glomerulonephritis ( $13.1 \pm 6.0$ ;  $P < 0.001$ ). In the post-infectious category, these comprised increases in monocytes and granulocytes with no significant increase in T cells or B cells. In the crescentic glomerulonephritides, intraglomerular monocytes only were significantly increased. There was no increase in the number of

glomerular T cells in any form of proliferative glomerulonephritis.

**Interstitial nephropathy.** The mean total glomerular leukocyte count in the interstitial nephropathy group was  $2.7 \pm 0.8$  cells per glomerular cross section. This was not significantly different from normal or non-proliferative glomerulonephritis, but was significantly less than the number seen in the proliferative glomerulonephritis group  $9.9 \pm 2.0$  ( $P < 0.05$ ).

**Glomerular immune reactant deposition (Table 3).** Immune reactant deposition was shown to be associated with altered glomerular leukocyte populations.

Monocyte accumulation was increased in the presence of IgG

Table 4. Intraglomerular leukocytes according to site of electron dense deposits

	Total leukocytes	P	Monocytes	P	Granulocytes	P	OKM1 + ve leukocytes	P	T Lymphocytes	P
Subepithelial deposits										
absent	5.1 ± 1.2 <sup>a</sup>	NS	2.0 ± 0.5	NS	1.9 ± 0.9	NS	3.2 ± 0.6	NS	0.4 ± 0.1	NS
present	10.9 ± 3.6		5.6 ± 2.4		4.3 ± 2.2		6.8 ± 2.4		0.5 ± 0.1	
Intra-membranous deposits										
absent	6.6 ± 1.4	NS	3.1 ± 0.9	NS	2.3 ± 0.8	NS	4.1 ± 0.9	NS	0.5 ± 0.1	<0.05
present	6.7 ± 4.1		1.5 ± 0.6		3.0 ± 2.7		3.8 ± 2.2		0.2 ± 0.1	
Subendothelial deposits										
absent	5.2 ± 1.2	<0.05	2.1 ± 0.6	NS	2.1 ± 0.9	NS	2.8 ± 0.5	<0.05	0.5 ± 0.1	NS
present	14.6 ± 4.9		4.6 ± 3.0		4.6 ± 2.6		11.8 ± 3.9		0.4 ± 0.2	
Mesangial deposits										
absent	5.9 ± 1.5	NS	2.5 ± 0.7	NS	2.5 ± 1.1	NS	3.1 ± 0.7	NS	0.5 ± 0.1	NS
present	8.4 ± 2.8		4.0 ± 2.3		2.5 ± 1.4		6.6 ± 2.1		0.4 ± 0.1	

Significance of difference between groups was assessed using Student's *t*-test<sup>a</sup> Expressed as cells/glomerular cross section (mean ± SEM)

Table 5. Interstitial leukocytes

	Number of patients	Total leukocytes	Monocytes		Granulocytes		T Lymphocytes		OKT4/OKT8 ratio	B Lymphocytes	
Normal	13	102 ± 18 <sup>a</sup>	34 ± 10	34% <sup>b</sup>	7.0 ± 2.5	7%	33 ± 14	33%	1.3 ± 0.5	2.5 ± 1.0	2%
Non-proliferative GN											
Non-spec.	19	203 ± 42	57 ± 15	28%	7.0 ± 1.5	3%	66 ± 15 <sup>f</sup>	32%	1.0 ± 0.4	6.0 ± 3.0	3%
Min. les.	4	78 ± 24	8 ± 3	10%	5.0 ± 5.0	6%	31 ± 12	40%	0.2 ± 0.1	NA <sup>c</sup>	NA
Membranous	13	219 ± 41 <sup>e</sup>	48 ± 15	22%	10.0 ± 3.5	5%	134 ± 30 <sup>f</sup>	61% <sup>e</sup>	0.5 ± 0.1	15.0 ± 5.0 <sup>e</sup>	7%
FGS	13	424 ± 74 <sup>f</sup>	145 ± 40	34%	13.0 ± 3.5	3%	207 ± 53 <sup>f</sup>	49%	1.9 ± 0.6	29 ± 14.0 <sup>e</sup>	7%
Diabetes	9	221 ± 40 <sup>e</sup>	49 ± 17	22%	9.0 ± 4.5	4%	198 ± 81 <sup>f</sup>	89% <sup>e</sup>	1.0 ± 0.4	4.0 ± 2.5	2%
Proliferative GN											
PINF	8	472 ± 122 <sup>f</sup>	124 ± 43 <sup>e</sup>	26%	41 ± 18 <sup>f</sup>	9%	183 ± 49 <sup>f</sup>	39%	1.4 ± 0.8	43 ± 15 <sup>f</sup>	9%
Idio.	6	582 ± 262 <sup>e</sup>	128 ± 63	22%	10 ± 5.0	2%	447 ± 199	77%	0.9 ± 0.6	NA	NA
IgA	18	565 ± 105 <sup>e</sup>	120 ± 32 <sup>f</sup>	21%	45 ± 26	8%	283 ± 59 <sup>e</sup>	50% <sup>e</sup>	1.4 ± 0.2	45 ± 18 <sup>f</sup>	8%
SLE	13	303 ± 62 <sup>f</sup>	79 ± 19	26%	8.0 ± 2.0	2%	215 ± 64 <sup>a</sup>	71% <sup>f</sup>	1.9 ± 0.7	28 ± 9.0 <sup>e</sup>	9%
Cresc.	14	790 ± 107 <sup>a</sup>	171 ± 32 <sup>f</sup>	22%	17.0 ± 5.0	2%	508 ± 101 <sup>a</sup>	64% <sup>f</sup>	1.0 ± 0.2	83 ± 30 <sup>e</sup>	10%
MPGN	8	690 ± 154 <sup>f</sup>	186 ± 33 <sup>e</sup>	27%	29 ± 11 <sup>e</sup>	4%	481 ± 127 <sup>e</sup>	70% <sup>e</sup>	1.4 ± 0.5	186 ± 58 <sup>f</sup>	27%
Focal	10	460 ± 82 <sup>f</sup>	96 ± 28 <sup>e</sup>	21%	11.0 ± 4.0	2%	289 ± 92 <sup>a</sup>	63% <sup>e</sup>	1.2 ± 0.6	30 ± 12 <sup>f</sup>	7%
Interst. neph.	10	1425 ± 447 <sup>f</sup>	359 ± 146 <sup>f</sup>	25%	72 ± 37 <sup>e</sup>	5%	861 ± 240 <sup>a</sup>	60% <sup>f</sup>	1.6 ± 0.3	174 ± 68 <sup>e</sup>	12%

<sup>a</sup> Expressed as cells per square millimeter<sup>b</sup> Percentage of total leukocytes (defined by common leukocyte marker, PHM-1)<sup>c</sup> No data available<sup>e</sup> *P* < 0.05<sup>f</sup> *P* < 0.01<sup>a</sup> *P* < 0.001

when all biopsies showing the presence of IgG on fluorescence microscopy were compared with those negative for IgG. In patients with crescentic nephritis, there was a marked increase in glomerular tuft leukocytes where glomerular IgG was demonstrated compared with those crescentic biopsies which did not demonstrate positive staining for IgG (IgG positive 24.4 ± 13.6, IgG negative 5.0 ± 0.7; *P* < 0.05).

Glomerular leukocyte numbers were significantly decreased in the presence of IgM.

There was no significant alteration in the intraglomerular leukocyte populations according to the presence or absence of IgA deposition.

There was a statistically significant increase in glomerular monocyte numbers in the presence of C<sub>3</sub> deposition.

Fibrin deposition was associated with a significant increase in the glomerular accumulation of total leukocytes. There was a concomitant increase in granulocyte accumulation. There was an increased number of glomerular tuft monocytes in the presence of fibrinogen deposition, but this did not reach statistical significance.

In all the above categories, no significant difference in the minimal numbers of T cells, B cells and NK cells was seen in the presence or absence of immune reactants.

*Leukocyte subpopulations related to electron-dense deposit*

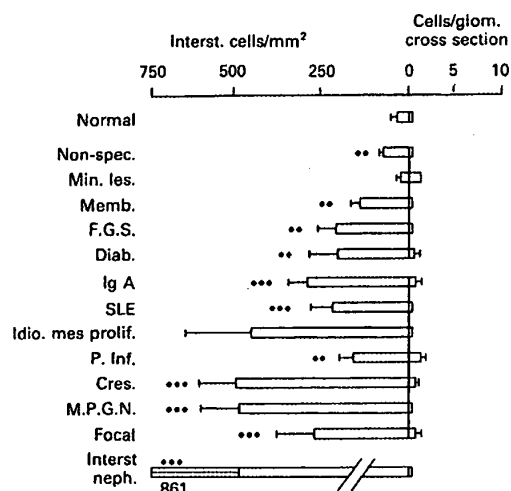


Fig. 2. T lymphocytes in the glomerulus and the interstitium. Although occasional T cells are seen in the glomeruli in some biopsies there is no significant increase in any form of glomerulonephritis. However, T lymphocytes constitute the predominant cell type in the interstitium in human glomerulonephritis with the exception of minimal change disease.

formation (Table 4). Glomerular leukocyte numbers were not related to the presence or absence of electron dense deposits in subepithelial, intramembranous or mesangial sites. However, there was a significant increase in infiltrating glomerular leukocytes associated with subendothelial deposits.

#### Interstitial leukocyte infiltration (Table 5).

Interstitial mononuclear cell infiltration was present in all forms of glomerulonephritis and was a prominent feature in those proliferative glomerulonephritides.

**Normal.** Leukocytes, as labelled by the monoclonal antibody to the leukocyte common antigen, were identified within the interstitium of normal renal tissue ( $102 \pm 18$  cells/mm<sup>2</sup>). Thirty-four per cent of these cells were monocytes, 33% T lymphocytes and granulocytes 7%. The mean in situ OKT4<sup>+</sup>/OKT8<sup>+</sup> ratio was  $1.3 \pm 0.5$ .

**Non-proliferative glomerulonephritis.** A significant increase in interstitial leukocytes above normal was seen in membranous nephropathy, focal glomerulosclerosis and diabetic nephropathy. There was no significant increase in interstitial monocytes or granulocytes seen in any of the non-proliferative glomerulonephritides. There was a small increase in B lymphocytes in membranous nephropathy and focal glomerulosclerosis. The predominant cell type was the T lymphocyte, with significant increases seen in membranous nephropathy, focal glomerulosclerosis, diabetic nephropathy, and even those biopsies which only showed mild, non-specific glomerular abnormalities.

The OKT4/OKT8 ratio for each disease state varied between 0.5 and 1.9, but the differences were not statistically different from the normal, renal interstitial OKT4/OKT8 ratio.

**Proliferative glomerulonephritis.** There was a significant increase in total interstitial leukocyte numbers in each of the seven types of proliferative glomerulonephritis. There was no alteration in the proportions of monocytes or granulocytes

comprising the interstitial infiltrate; however, in some disease states there was a significant increase in absolute numbers. Compared with normal interstitium, the number of monocytes was significantly increased in post-infectious glomerulonephritis, IgA nephropathy, membrano-proliferative glomerulonephritis and focal proliferative glomerulonephritis. Granulocytes were identified within the interstitium only in small numbers in all disease states, but were significantly increased in post-infectious glomerulonephritis and membrano-proliferative glomerulonephritis when compared with normal.

The predominant cell in the interstitial infiltrate was the T lymphocyte. There was a marked increase in interstitial T lymphocytes in all forms of proliferative glomerulonephritis. The OKT4<sup>+</sup>/OKT8<sup>+</sup> ratio ranged from 0.9 to 1.9 but was not significantly altered from the normal ratio in any disease state.

**Interstitial nephritis.** All cell types were significantly increased in number in the interstitial nephritis group, with a marked increase in the proportion of T lymphocytes—60% ( $P < 0.01$ ). Comparison of cell populations in interstitial nephritis and proliferative glomerulonephritis revealed no significant differences apart from a decrease in monocyte and granulocyte numbers in lupus nephritis and a decrease in B lymphocytes in IgA nephropathy.

**Comparison between T lymphocytes in the glomerulus and interstitium (Fig. 2).** T lymphocytes within the glomerulus were not identified in greater numbers than normal in any form of glomerulonephritis, whereas T lymphocytes comprised the greater portion of the interstitial inflammatory infiltrate in all forms of GN except minimal change nephropathy.

**Renal function, urinary indices and leukocyte populations (Table 6).** There was a clear correlation between the number of interstitial total leukocytes and impairment of renal function as measured by both the serum creatinine ( $r = 0.60$ ;  $P = 0.001$ ) and the creatinine clearance ( $r = -0.58$ ;  $P = 0.001$ ).

The proportions of monocytes, lymphocytes and granulocytes comprising the interstitial infiltrate were examined according to the degree of renal impairment. There was no significant change in cell proportions (by analysis of variance) as renal function deteriorated.

The correlation between infiltrating interstitial cells and renal functional impairment was strongest for T cells ( $r = 0.54$ ;  $P = 0.001$ ) and weaker, yet significant for monocytes ( $r = 0.46$ ;  $P = 0.001$ ) and B lymphocytes ( $r = 0.33$ ;  $P = 0.001$ ).

There was no good correlation between proteinuria, urinary red cell excretion and glomerular leukocyte accumulation.

**Relationship between intraglomerular monocytes and interstitial leukocyte infiltration.** There was a significant correlation between intraglomerular monocytes and the total number of interstitial leukocytes ( $r = 0.42$ ;  $P = 0.001$ ). This correlation was also seen particularly with interstitial T lymphocytes ( $r = 0.39$ ;  $P = 0.001$ ) and B lymphocytes ( $r = 0.44$ ;  $P = 0.001$ ).

#### Discussion

With the advent of monoclonal antibodies as specific markers of functional subpopulations of leukocytes [9, 15], and the development of suitable and sensitive techniques to localize such markers within tissues [8], it has recently become feasible to identify and localize infiltrating leukocytes in situ within the kidney [6]. The leukocyte profile within diseased glomeruli has been delineated for many forms of glomerulonephritis [20] and

Table 6. Correlation between interstitial leukocytes and renal function and between glomerular leukocytes and renal function

	Site	Total leukocytes	Monocytes	Granulocytes	B Lymphocytes	T Lymphocytes	OKT4/OKT8
Serum creatinine	I	$r = 0.60^c$	$r = 0.46^c$	NS	$r = 0.33^c$	$r = 0.54^c$	NS
	G	$r = 0.27^b$	$r = 0.32^b$	$r = 0.25^b$		NS	
Creatinine clearance	I	$r = 0.58^c$	$r = -0.43^c$		NS	$r = -0.32^c$	$r = 0.52^c$
	G	$r = -0.25^b$	$r = -0.29^a$	$r = -0.21^a$		NS	
Urinary protein excretion	I	NS	NS	NS	NS	$r = 0.21^b$	NS
	G	$r = -0.18^a$	NS	NS		NS	
Urinary red cell excretion	I	NS	NS	NS	NS	NS	NS
	G	$r = 0.24^b$	NS	NS		$r = 0.22^a$	

Abbreviations are: I, interstitial cells; G, glomerular cells.

<sup>a</sup>  $P < 0.05$

<sup>b</sup>  $P < 0.01$

<sup>c</sup>  $P < 0.001$

this study has extended these previous observations, determined the relationship with glomerular immune reactants, and emphasized the importance of the interstitial changes that occur in most types of human glomerulonephritis and which have not been fully appreciated in the past.

The normal leukocytic component of both glomeruli and interstitium has been determined. There is a consistent small population of cells of monocyte-macrophage phenotype within the glomerulus itself. Such cells have been recognized by tissue culture of normal human and animal glomeruli [21, 22], and may be analogous to the resident Ia<sup>+</sup> glomerular cell with the properties of mononuclear phagocytes recently described by Schreiner et al in rats [23]. T cells were rarely seen within the normal glomerulus and B cells, NK cells and polymorphonuclear leukocytes were never identified. In contrast, the normal interstitium contained a significant number of leukocytes consisting predominantly of monocytes and T cells but with occasional B cells and granulocytes also present.

In several forms of human non-proliferative glomerulonephritis, there is diminution in numbers of monocytes in glomeruli, an observation not previously described. This reduction in resident glomerular macrophages was seen in those biopsies showing minor non-specific glomerular abnormalities, minimal lesion nephropathy, membranous nephropathy and focal glomerulosclerosis. The mechanism of this alteration is not clear. Our results demonstrate that the presence of IgM appeared to mitigate against the accumulation of glomerular mononuclear cells when examined over the whole spectrum of glomerulonephritides. However, this would not explain the situation in membranous nephropathy where marked subepithelial IgG deposition was present, and might from our overall correlations be expected to be associated with increased monocyte accumulation. Indeed, mononuclear phagocytes are known to express receptors for the Fc portion of IgG monomers and complexes [24].

Glomerular leukocyte (monocytes and granulocytes) accumulation was shown to be significantly increased in the presence of subendothelial deposits on electron microscopy. This finding accords with that of Magil, Wadsworth and Loewen [25] who found increased glomerular monocytes (using non-specific esterase staining) in the presence of subendothelial deposits. They postulated that the proximity of the deposits to the

capillary lumen together with chemotactic components of complement led to the monocyte accumulation.

Whether or not glomerular macrophage accumulation is involved in cellular immune reactions, macrophages do have a pathogenic role. In both human and experimental glomerulonephritis macrophages in nephritic glomeruli have expressed the procoagulant tissue factor which triggers the extrinsic pathway of coagulation leading to fibrin deposition [26, 27]. Macrophages also play a role in extracapillary crescent formation [6].

The paucity of infiltrating glomerular leukocytes, particularly T cells, glomerulonephritis other than crescentic glomerulonephritis and post-infectious glomerulonephritis, contrasts markedly with the extensive mononuclear leukocytic infiltrate, (including T cell) in the renal interstitium in most types of glomerulonephritis. Recent monoclonal antibody analysis of the classical cutaneous delayed-type hypersensitivity (DTH) reaction has demonstrated a response characterized by T lymphocytes and monocytes, particularly related to small blood vessels, early enrichment of T4 cells and later infiltration by further T lymphocytes and monocytes [28]. This pattern of mononuclear cell infiltration is very similar to that seen in the interstitium in this study and in other studies [29, 30], suggesting that delayed type hypersensitivity may play a role in the immunopathogenesis of the interstitial renal damage in glomerulonephritis.

Whether the interstitial inflammation is initiated by the same antigen which initiates the glomerular injury or whether it is a reaction to unmasked antigen leaking from damaged tubules and reacting with circulating antibody in the peritubular capillaries [31], remains unresolved. In experimental models of tubulointerstitial nephritis, endogenous antigens have been shown to induce a cell-mediated, mononuclear interstitial infiltrate. This has been shown in Lewis rats which do not possess the antigen to which anti-TBM antibodies are raised [32], in Heymann nephritis [33] and in rabbits immunized with homologous kidney [31]. Exogenous antigens have also been shown to elicit a DTH reaction when injected into the renal interstitium [34]. Anti-TBM antibodies have been demonstrated in 70% of patients with crescentic glomerulonephritis due to anti-GBM disease and granular deposits of immunoglobulin in the interstitium, presumably representing immune complexes, have been demonstrated in a proportion of patients with lupus

nephritis [35]. However, in most glomerulonephritides no such interstitial immune reactants are present and so some other explanation for the interstitial leukocytic accumulation, must be sought.

Although increased OKT4<sup>+</sup>:OKT8<sup>+</sup> ratios in the circulation have been demonstrated in patients with IgA nephropathy, membranous nephropathy, and focal glomeruloclerosis [36], there was no demonstrable alteration from the normal OKT4<sup>+</sup>:OKT8<sup>+</sup> ratio examined in situ in the interstitium. Hence, whatever the immunoregulatory process leading to these observations, it cannot be said that the in situ population of lymphocytes merely reflects changes in the circulating lymphocyte population.

The density of mononuclear leukocytic infiltration in the interstitium, but not the glomerulus, correlates with the degree of renal impairment. This observation agrees with previous data which suggests that renal function is much more closely correlated with interstitial fibrosis than it is with glomerular changes in patients with glomerulonephritis [37-39]. It may well be that the interstitial leukocyte infiltration per se is the underlying determinant of the progression of renal failure in glomerulonephritis, rather than the glomerular changes.

The current study clearly demonstrates that the classical mononuclear participants in a cell-mediated immune reaction are present in the kidney in patients with glomerulonephritis. We believe that this interstitial leukocytic infiltration is an important component of most forms of glomerulonephritis and may well play a major role in both the immune pathogenesis and the progression of glomerulonephritis.

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# Nonsteroidal Anti-Inflammatory Drugs: Effects on Kidney Function

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are capable of inducing a variety of renal function abnormalities, particularly in high-risk patients with decreased renal blood perfusion who depend on prostaglandin synthesis to maintain normal renal function. Fluid retention is the most common NSAID-related renal complication, occurring to some degree in virtually all exposed individuals; however, clinically detectable edema occurs in less than 5% of patients and is readily reversible on discontinuation of the NSAID. Other electrolyte complications, notably hyperkalemia, are seen infrequently and occur in specific at-risk patients. The next most worrisome complication is acute deterioration of renal function, which occurs in high-risk patients and is also reversible. Nephrotic syndrome with interstitial nephritis is a rare problem of NSAID use and is reversible. Papillary necrosis is the only permanent complication of NSAIDs and is very rare. Altogether, these renal function abnormalities, with the exception of mild fluid retention, are clinically detectable in approximately 1% of exposed patients. Given the number of patients who take NSAIDs on a prescription or over-the-counter basis, the absolute number of at-risk patients is relatively large. Consequently, an appreciation for the risk factors and pathophysiology of NSAID-induced renal function abnormalities is required for optimal use of these drugs.

Approximately 1-5 of persons who are exposed to a nonsteroidal anti-inflammatory drug (NSAID) will manifest one of a variety of renal function abnormalities. Although this percentage appears relatively low, the number of at-risk individuals is enormous because of the current use profile of NSAIDs, either as prescription or over-the-counter drugs. One in seven Americans is likely to be treated with an NSAID for a chronic rheumatologic disorder. If patients who take NSAIDs for acute problems are considered, the exposure rate will be even higher. Thus, of the 50 million Americans expected to use NSAIDs intermittently or routinely this year, at least 500,000 are likely to develop some degree of renal functional abnormality.

In descending order of frequency, the primary NSAID-related renal abnormalities are 1) fluid and electrolyte disturbances, 2) acute deterioration of renal function, 3) nephrotic syndrome with intersti-

tial nephritis, and 4) papillary necrosis (Table I). Sodium chloride and water retention, the most commonly encountered renal effect of NSAID use, occurs to some degree in virtually all exposed persons but results in clinically detectable edema in less than 5% of patients. This rate is probably higher in selected at-risk patients. NSAID-induced fluid retention is typically benign, reversible on discontinuation of the NSAID, and easily managed in patients who require treatment. Other electrolyte abnormalities are also induced by NSAIDs, the most important of which is potassium retention and hyperkalemia. A high-risk group can also be identified for this electrolyte abnormality.

From the clinical point of view, the most worrisome renal side effect of NSAIDs is hemodynamically mediated acute renal failure, which occurs in individuals with pre-existing reduced renal blood perfusion. Ordinarily, the kidneys of such at-risk patients produce vasodilatory prostaglandins to maintain renal perfusion and function. The inhibitory effects of NSAIDs on renal prostaglandin production lead to acute, reversible renal failure in these patient. Acute deterioration of renal function occurs in 0.5 to 1% of patients who take NSAIDs on a chronic basis.

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# NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

TABLE I

Documented Renal Effects of Nonsteroidal Anti-Inflammatory Drugs

Drug Class	Generic Name	Brand Name/ Manufacturer	Renal Effects*				
			Edema	↑K	ARF	NS	PN
Salicylates	Aspirin	(various)	CI		CI		CI
	Diflunisal	Dolobid/Merck	CI		CI	CI	An
Propionic acids	Ibuprofen	Motrin/Upjohn	CI	CI	CI	CI	CI
	Naproxen	Naprosyn/Syntex	CI		CI	CI	An
	Fenoprofen calcium	Nalfon/Lilly	CI		CI	CI	CI
	Ketoprofen	Orudis/Wyeth-Ayerst	CI		CI	CI	
Indolacetic acids	Flurbiprofen	Ansaid/Upjohn	CI	CI	CI	CI†	An
	Indomethacin	Indocin/Merck	CI	CI	CI	CI	CI‡
	Sulindac	Clinoril/Merck	CI	CI	CI	CI	An
	Tolmetin	Tolectin/McNeil	CI		CI	CI	An
	Diclofenac	Voltaren/Ciba-Geigy	CI		CI	CI	CI
Anthranilic acids	Meclofenamate sodium	Meclofen/Parke-Davis	CI		CI	CI	An
	Mefenamic acid	Ponstel/Parke-Davis	CI		CI	CI	CI
Pyrazolones	Phenylbutazone	Butazolidin/Ciba-Geigy	CI		CI	CI	CI
Oxicams	Piroxicam	Feldene/Pfizer	CI	CI	CI	CI	CI

\* ARF = acute renal failure; NS = interstitial nephritis and nephrotic syndrome; PN = papillary necrosis; ↑K = hyperkalemia; CI = reported in clinical studies; An = described in studies in animals (but not in humans).

† Causes interstitial nephritis without nephrotic syndrome.

‡ Reported in combination with phenylbutazone.

(Adapted from Clive and Stoff,<sup>1</sup> with permission.)

The nephrotic syndrome, with associated interstitial nephritis, is seen on rare occasions. Once again, it is reversible on discontinuation of the NSAID in question.

According to the respective manufacturers' prescribing information, chronic administration of nearly all NSAIDs produces papillary necrosis in laboratory animals; and a few clinical case reports of papillary necrosis can be found in the recent medical literature. Within the framework of our present understanding of NSAID effects on the kidney, this appears to be the only irreversible form of renal toxicity.

Many of the renal abnormalities that are encountered as a result of NSAID use can be attributed to the action of these drugs on prostaglandins. Hence, a brief overview of the interactions between prostaglandins and renal function will be presented, followed by an analysis of the pathophysiology, clinical manifestations, patient risk factors, and preventive approaches to NSAID-induced renal syndromes.

## THE PROSTAGLANDIN PATHWAY

Prostaglandins are ubiquitous substances that influence renal function along with a variety of other body systems.<sup>1,2</sup> Conceptually, they may be considered local hormones or "autocoids" because they act in a paracrine or autocrine fashion. Biologic activity is limited to the site of action by the short half-life of

prostaglandins in circulation. In addition, prostaglandins are not stored in tissue, but are synthesized on demand.

Prostaglandins are derived from phospholipids by a common pathway (Figure 1). Phospholipids, of course, are widely distributed in cell membranes throughout the body. The most important precursor for prostaglandins is arachidonic acid. Cyclooxygenase is the catalyst for oxygenation of arachidonic acid, which is the step that is inhibited by NSAIDs. The interaction between aspirin and cyclooxygenase (acetylation) is irreversible, whereas that with other NSAIDs is reversible.

Arachidonic acid can also be metabolized to other mediators, depending on the cell type. For example, lipoxygenase catalyzes the production of leukotrienes, and mixed-function oxygenases catalyze the production of epoxyeicosatrienoic acids. Collectively, these oxygenated metabolites of arachidonic acid are known as eicosanoids because of their origin from a 20-carbon (eicosa-) polyunsaturated acid.<sup>3</sup>

Continuing along the common pathway (Figure 1), oxygenation of arachidonic acid results in production of prostaglandin G<sub>2</sub>, which is converted to prostaglandin H<sub>2</sub> by hydroperoxidase and loss of a free radical. At this point, metabolism becomes highly specific for individual cell types, although many, if not all, of the metabolites are produced in the kidney. Prostaglandin E<sub>2</sub> is a vasodilator, which, in the kidney, promotes diuresis and natriuresis. Prostaglan-



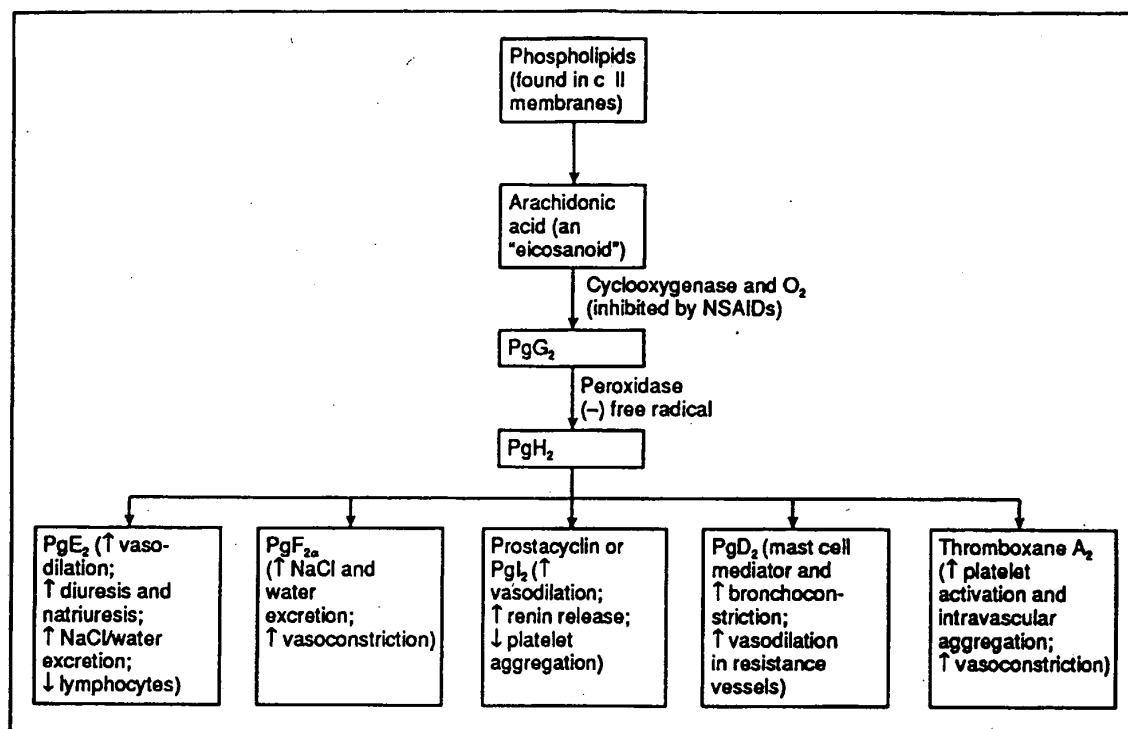


Figure 1. Prostaglandin pathway (and prostanoid functions). Pg = prostaglandin; ↑ = stimulate or increase; ↓ = inhibit or decrease.<sup>2,3</sup>

din E<sub>2</sub> also inhibits lymphocytes and other cells that are involved in inflammation and allergic responses, which, as will be discussed later, may play a role in some NSAID-induced renal syndromes. Prostaglandin F<sub>2α</sub> enhances excretion of sodium chloride and water. Prostacyclin, also known as prostaglandin I<sub>2</sub>, has a wide variety of actions including vasodilation, renin release, and inhibition of platelet aggregation. Prostaglandin D<sub>2</sub> is a vasodilator of peripheral resistance vessels but is better known for its association with mast cell activation and bronchoconstriction. Thromboxane A<sub>2</sub> is the principal metabolite of prostaglandin H<sub>2</sub> in platelets and can act as a major vasoconstrictor within the kidney. These pharmacologically active metabolites of prostaglandin H<sub>2</sub> are collectively known as prostanoids.<sup>3</sup>

#### PROSTAGLANDIN EFFECTS ON RENAL FUNCTION

Given the diversity of cell populations within the kidney and their various functions, the complexity of the interactions between prostaglandins and renal function is not unexpected. Prostaglandins are involved in renin release, local vascular tone, regional

circulation, sodium and water homeostasis, and potassium balance (Table II). The following sections describe these diverse effects. Detailed overviews of these interactions can be found in excellent reviews by Patrono and Dunn<sup>2</sup> and Oates and colleagues.<sup>3</sup>

An important caveat in the following sections is that prostaglandins are not primary mediators of basal renal function in normal individuals. Prostaglandins typically operate in conjunction with a variety of other mediators, which, even in the absence of prostaglandins, can preserve homeostasis. Prostaglandin production is increased as needed in response to stress (e.g., decreased renal blood flow or blood volume). Thus, inhibition of prostaglandin function by NSAIDs is more likely to cause complications in at-risk patients with decreased renal blood perfusion than in the otherwise normal subject whose prostaglandins are merely one of many factors contributing to homeostasis.

#### Renin Release

Prostaglandins stimulate renin release, which plays an important role in the regulation of arterial blood

TABLE II

Principal Renal Sites of Prostaglandin Synthesis and Major Actions

Site	Eicosanoid	Action
Vasculature	Prostaglandins $I_2$ and $D_2$	Vasodilation
Glomerulus	Prostaglandins $I_2$ and $E_2$	Maintain GFR
Collecting tubule	Thromboxane $A_2$	Reduce GFR
	Prostaglandins $E_2$ and $F_{2\alpha}$	Enhance excretion of sodium chloride and water
Medullary interstitial cells	Prostaglandin $E_2$	Vasodilation and natriuresis-diuresis

(Adapted from Patrono and Dunn<sup>2</sup>, with permission.)

pressure, blood volume, and electrolyte balance. Prostaglandins can act independently or synergistically with the  $\beta$ -adrenergic system.<sup>4</sup> Although the exact prostanoid mediator is not yet known, it is likely that prostacyclin is synthesized in response to a change in arteriole pressure or chloride reabsorption in the macula densa of the nephron.<sup>3</sup>

#### Local Vascular Tone

Prostanoids are one of several local mediators that govern vascular tone through their actions on norepinephrine release at peripheral nerve endings. Prostaglandins  $E_2$  and  $D_2$  and, to a lesser extent, prostacyclin promote vasodilation by inhibiting norepinephrine release. Prostaglandin  $E_2$  also antagonizes the effects of angiotensin II, a powerful vasopressor, on the neuroeffector junction. Conversely, prostaglandin  $F_{2\alpha}$  and thromboxane  $A_2$  are vasoconstrictors.<sup>3</sup>

#### Regional Circulation

Prostanoids contribute to regional circulation via their influence on local vascular tone. Under normal conditions, prostanoids do not regulate renal perfusion per se. However, certain conditions such as decreased renal blood flow are associated with the production of vasodilatory prostaglandins. Prostaglandin  $E_2$ , prostacyclin, and prostaglandin  $D_2$  shift regional blood flow from cortical to juxtamedullary nephrons.<sup>3</sup>

#### Sodium and Water Homeostasis

All prostanoids are capable of acting in the renal cortex to regulate sodium and water homeostasis; however, prostanoids are only one of many factors that share this function.<sup>3</sup> Prostaglandins  $E_2$  and  $D_2$ , prostacyclin, and, to a lesser extent, prostaglandin  $F_{2\alpha}$  increase the rate of salt and water excretion. Prostaglandin  $E_2$  inhibits sodium chloride transport in the thick ascending limb of the loop of Henle and the collecting duct.<sup>5,6</sup> In addition, prostaglandins antagonize the effects of antidiuretic hormone.<sup>7,8</sup>

Prostanoids do not have a direct effect on glomerular filtration rate; however, vasodilation associated with prostaglandin  $E_2$ , prostacyclin, and prostaglandin  $D_2$  increases renal blood flow, and, as previously mentioned, shunts blood flow from the cortical to juxtamedullary nephrons. The net result is enhanced diuresis and natriuresis due to reduced medullary hypertonicity and increased interstitial pressure.<sup>3</sup>

#### Potassium Balance

Prostanoids indirectly lower potassium by their effects on glomerular filtration and renin.<sup>3</sup> As previously mentioned, vasodilatory prostaglandins increase renal blood flow. This may enhance the direct intratubular delivery of potassium into the distal nephron for excretion. Alternatively, this may serve to quantitatively increase sodium delivery into the distal nephron with resultant reabsorption of sodium in exchange for potassium, which is then excreted in the urine. Secondly, prostacyclin is believed to promote renin release. Activation of the renin-angiotensin pathway ultimately causes aldosterone to stimulate potassium excretion in the distal convoluted tubule and collecting duct. However, potassium balance is also regulated by a number of other factors such as insulin and the  $\beta$ -adrenergic system.

#### FLUID AND ELECTROLYTE DISTURBANCES

##### Sodium and Water Retention

The most common and universal renal complications of NSAIDs are sodium retention and edema. According to prescribing information accompanying nearly all NSAIDs, edema occurs in at least 3% of patients. The incidence is probably higher in patients who take therapeutic doses over prolonged periods. The onset of fluid retention usually occurs early in the course of therapy and can be dramatic as

illustrated by the 15-kg weight gain in a 70-year-old man who took ibuprofen for only 17 days.<sup>9</sup>

Occasionally, the patient may retain water in excess of sodium. Severe, reversible hyponatremia (118  $\mu\text{mol Na}^+/\text{L}$ ) occurred in a patient who took ibuprofen for only 3 days. This patient had underlying renal impairment ( $\text{CrCl}$  12 mL/min).<sup>10</sup>

The multiple mechanisms by which NSAIDs interfere with water and sodium metabolism may explain the frequency of this complication. As previously mentioned, NSAIDs have the potential to disrupt diuresis and natriuresis by interfering with prostaglandin-mediated sodium chloride transport, antidiuretic hormone, and distribution of blood flow from cortical to juxtamedullary nephrons.<sup>1,3</sup> The hypothesis for the pathogenesis of the nephrotic syndrome is also operative in this situation. By shunting arachidonic acid metabolism from prostaglandins to lipoxygenase products, NSAIDs may favor production of eicosanoid derivatives that increase capillary permeability.<sup>1</sup>

### Hyperkalemia

Hyperkalemia is an unusual complication of NSAIDs, presumably because of the multiplicity of factors that are capable of maintaining potassium balance, even in the absence of prostaglandins. Hyperkalemia is more likely to occur in patients with pre-existing renal impairment,<sup>11,12</sup> cardiac failure,<sup>13</sup> diabetes,<sup>12</sup> or multiple myeloma<sup>14</sup> or in patients who receive potassium supplementation,<sup>15</sup> potassium-sparing diuretics,<sup>16</sup> or angiotensin-converting enzyme (ACE) inhibitors. Indomethacin appears to be the major NSAID associated with this complication and has produced hyperkalemia in patients without apparent risk factors.<sup>17</sup> Thus, indomethacin may have a direct effect on the cellular uptake of potassium,<sup>18</sup> in addition to the known effects of NSAIDs on potassium delivery to the distal tubule as well as on the renin-angiotensin and aldosterone pathways.

NSAID-induced hyperkalemia often occurs in the setting of NSAID-induced acute renal deterioration or worsening of underlying renal impairment. However, the severity of hyperkalemia can be disproportionate to that of renal impairment. For example, Tan and colleagues reported a patient who was treated with indomethacin and had a serum potassium of 6.2 mEq/L in spite of only mildly abnormal renal function.<sup>19</sup> In this patient, plasma renin and aldosterone levels were suppressed and did not respond to furosemide or postural changes. Urinary prostaglandin  $\text{E}_2$  was also suppressed. Discontinuation of indomethacin resulted in normalization of po-

tassium, prostaglandin  $\text{E}_2$ , and a rebound of renin and aldosterone.

## ACUTE DETERIORATION OF RENAL FUNCTION

### Role of Prostanoids in Maintaining Renal Blood Flow

Although NSAIDs do not impair glomerular filtration in normal individuals,<sup>20,21</sup> acute renal decompensation may occur in at-risk patients with various extrarenal or renal disease processes that lead to decreased renal perfusion (Table III). Renal prostaglandins play an important role in the maintenance of homeostasis in these patients, so drug-induced disruption of counter-regulatory mechanisms can produce clinically important and even severe renal functional deterioration.<sup>2,3</sup>

Acute renal deterioration in this setting can be attributed to the interruption of the delicate balance between hormonally mediated pressor mechanisms and prostaglandin-related vasodilatory effects (Figure 2). In at-risk patients, volume contraction triggers pressor responses via adrenergic and renin-angiotensin pathways. Ordinarily, vasodilatory renal prostaglandins counterbalance the vasoconstrictive effects of norepinephrine and angiotensin II. The addition of NSAIDs increases the risk of azotemia and possibly ischemic damage to the kidney by removing the protective effects of vasodilatory prostaglandins and allowing unopposed vasoconstriction.

### Clinical Features of Acute Renal Failure

Initially, this NSAID-induced renal syndrome is of moderate severity and is characterized by increasing BUN, creatinine, potassium, and weight with decreasing urine output. NSAID-induced acute renal failure is usually reversible over 2 to 7 days after discontinuation of therapy; however, morbid consequences can occur if the diagnosis is not recognized early. Continued NSAID therapy in the setting of de-

TABLE III

#### At-Risk Patients for NSAID-Induced Acute Renal Failure

Severe heart disease (congestive heart failure)
Severe liver disease (cirrhosis)
Nephrotic syndrome (chronic renal disease)
Elderly population
Dehydration (protracted)

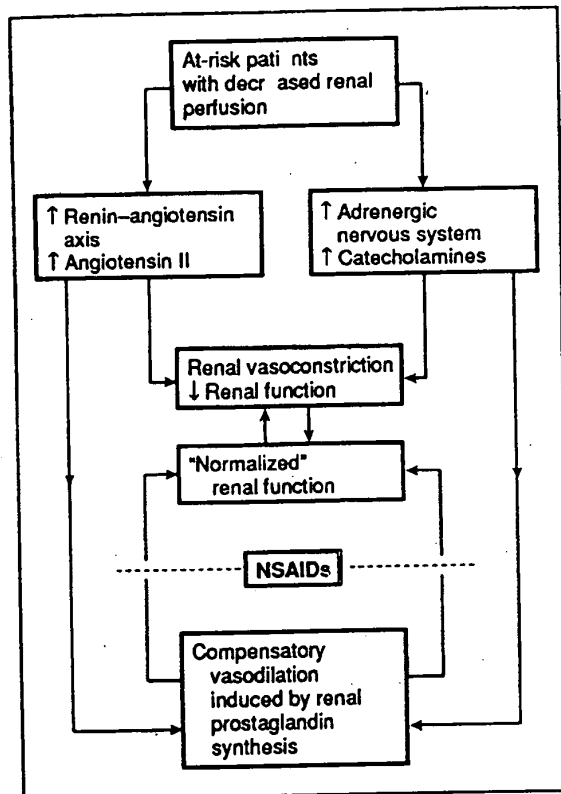


Figure 2. Mechanism by which NSAIDs disrupt the compensatory vasodilation response of renal prostaglandins to vasoconstrictor hormones in patients with prerenal conditions. A solid line (—) indicates stimulation; a dashed line (---) indicates inhibition.

teriorating renal function may progress rapidly to the point wherein dialysis support is required.<sup>22</sup> Despite this profound level of renal functional impairment, the kidney will nonetheless recover several days to weeks after discontinuation of the NSAID. Development of this type of "total" renal failure, which is often inappropriately designated as "acute tubular necrosis," represents the extreme end of the spectrum of hemodynamic insult rather than a separate clinical entity.

#### Risk Factors for Acute Renal Failure

The risk of acute renal deterioration is highest in patients with liver disease, pre-existing renal impairment, cardiac failure, protracted volume contraction due to diuretic therapy or intercurrent disease, or old age. NSAID-induced renal decompensation has been well documented in patients with cirrhosis, par-

ticularly when ascites is present.<sup>3</sup> Urinary excretion of prostaglandin  $E_2$ , prostacyclin metabolites, and thromboxane  $A_2$  is increased in these patients.<sup>23,24</sup> An analogous situation exists in patients with underlying congestive heart failure,<sup>25</sup> nephrotic syndrome,<sup>26,27</sup> or lupus nephritis.<sup>28,29</sup>

Patients with chronic renal impairment are at increased risk of NSAID-induced renal failure because of inadequate renal prostaglandin production. We documented NSAID-induced acute renal failure in patients with asymptomatic mild, but chronic, renal failure (serum creatinine between 1.5 and 3.0 mg/dL).<sup>30</sup> Baseline excretion of urinary prostaglandin  $E_2$  and 6-keto-prostaglandin  $F_{1\alpha}$  was quantitatively lower in the individuals who developed NSAID-induced renal decompensation than in those who did not, and ibuprofen proved to be more problematic than either piroxicam or sulindac. On initiation of ibuprofen, urinary prostaglandin excretion fell in all patients, but trough concentrations were quantitatively lower in the subset of patients who experienced acute renal failure.

Volume contraction due to diuretic therapy or an intercurrent disease that results in dehydration represents another important risk factor for the development of NSAID-induced acute deterioration of renal function.<sup>22,31,32</sup> Elderly patients are also at increased risk. We estimate that age of 80 years or greater is an independent risk factor because the physiology of ageing within the kidney results in 50% loss of function in 50% of the population at age 80, primarily as a result of the progression of arteriolonephrosclerosis.

#### Pharmacodynamics of Acute Renal Failure

NSAID-induced acute renal decompensation is a pharmacologically predictable phenomenon that occurs in a dose-related fashion. In our triple-crossover study of 12 women with mild renal failure, ibuprofen (800 mg three times daily) was discontinued on day 8 because of worsening renal function ( $\geq 1.5$  mg/dL increase in serum creatinine) or hyperkalemia (potassium  $\geq 6$  mEq/mL) in 3 patients. When these patients were rechallenged at a 50% lower dose of ibuprofen, two patients again had evidence of acute renal deterioration.<sup>30</sup>

Another important finding in our study was the time of onset of acute renal decompensation.<sup>30</sup> Ibuprofen-induced renal failure occurred rapidly (within days), but piroxicam and sulindac did not cause renal deterioration during the 11-day treatment period. A pharmacokinetic analysis in these patients provides insight. Ibuprofen, which has a short elimination half-life, reached maximum serum

concentrations quickly. In contrast, piroxicam and sulindac have longer half-lives and continued to accumulate throughout the treatment period. These findings are consistent with basic pharmacologic principles and suggest that NSAIDs having short elimination half-lives will reach steady state and exert maximum pharmacologic effects before NSAIDs having longer half-lives.

#### "Renal Sparing" NSAIDs — ?

Although all NSAIDs have the potential to induce acute renal impairment, some quantitative differences may exist. Sulindac has been hypothesized to be renal sparing, possibly because of its unusual metabolic pathway.<sup>33</sup> The parent compound, sulindac sulfoxide, is an inactive prodrug that undergoes hepatic metabolism to sulindac sulfide, which is the metabolite that exerts anti-inflammatory activity. Sulindac sulfoxide is also metabolized to a much lesser extent to an inactive metabolite, sulindac sulfone. It has been hypothesized that, within the kidney, sulindac sulfide is reversibly oxidized to the inactive parent compound, sulindac sulfoxide, such that renal prostaglandin production would not be influenced.

In clinical studies, urinary prostaglandin levels and renal effects were unchanged in patients with normal renal function<sup>34,35</sup> and states of proteinuria.<sup>36</sup> However, the duration of sulindac in these studies may have been insufficient to appreciate the full pharmacologic effect of sulindac. NSAID-induced changes may not have been detectable because of the presence of only very mild renal impairment or absence of renal failure altogether in these studies. Longer courses of sulindac in patients with slightly more severe renal impairment have been associated with statistically significant reductions in urinary prostaglandins<sup>30</sup> and glomerular filtration rate.<sup>37</sup>

The ability of sulindac to inhibit prostaglandin synthesis and impair renal function has been confirmed in a different high-risk group, namely patients with hepatic cirrhosis and ascites.<sup>38</sup> We have also identified the development of profound acute renal failure in high-risk patients who received sulindac for several days to weeks. Collectively, these clinical experiences indicate the need for cautious and timely monitoring of high-risk patients who receive NSAIDs.

#### NEPHROTIC SYNDROME WITH INTERSTITIAL NEPHRITIS

NSAIDs also cause another type of renal dysfunction that is associated with various levels of functional

impairment and characterized by the development of the nephrotic syndrome with interstitial nephritis.<sup>1,22,39,40</sup> The clinical features, absence of risk factors, and pathophysiology distinguish this from other NSAID-induced renal syndromes and from classic drug-induced allergic interstitial nephritis.

The features of this NSAID-induced renal syndrome are variable. The patient may experience edema, oliguria, and/or foamy urine.<sup>41</sup> Systemic signs of allergic interstitial nephritis such as fever, drug rash, peripheral eosinophilia, and eosinophiluria are generally absent.<sup>1,22,40,41</sup> The urine sediment contains microscopic hematuria and pyuria.<sup>1,41</sup> Proteinuria typically is in the nephrotic range.<sup>1,39</sup> We have noted that renal functional deterioration can range from minimal to severe.

Characteristically, this form of nephrotic syndrome consists of minimal change glomerulonephritis with interstitial nephritis, which is an unusual combination of histologic findings. NSAID-induced nephrotic syndrome without interstitial disease is rare but has been reported in a handful of patients who took fenoprofen, sulindac, or diclofenac. Conversely, interstitial disease without nephrosis has been reported in a few patients, but this may, in fact, represent allergic interstitial nephritis.<sup>41</sup>

In spite of nephrotic-range proteinuria, the most impressive histopathologic findings involve the interstitium and tubules. A focal diffuse inflammatory infiltrate can be found around the proximal and distal tubules. We reported that the infiltrate primarily consisted of cytotoxic T lymphocytes but also contained other T cells, B cells, and plasma cells.<sup>39</sup> Changes in the glomeruli were minimal and resembled those of minimal change glomerulonephritis with marked epithelial-foot process fusion. Other investigators have reported similar findings.<sup>1,22,41,42</sup>

The onset of NSAID-induced nephrotic syndrome is usually delayed, having a mean time of onset of 5.4 months after initiation of NSAID therapy<sup>40</sup> and ranging from 2 weeks to 18 months.<sup>1</sup> NSAID-induced nephrotic syndrome is usually reversible 1 month to 1 year after discontinuation of NSAID therapy. During the recovery period, some patients may require dialysis. Corticosteroids have been used empirically, but it is not clear whether they hasten recovery.<sup>1,22,39</sup> If proteinuria does not significantly remit within 2 weeks after discontinuation of the NSAID, we recommend a standard, 2-month trial of corticosteroid therapy as would be employed in a nephrotic adult with idiopathic minimal change or membranous glomerulonephritis.

Risk factors are not well understood. Underlying renal impairment does not appear to be a risk factor. Old age has been suggested as a risk factor,<sup>22,40</sup> but

this may also be a reflection of the usual candidate for chronic NSAID therapy. The syndrome has been more commonly reported with fenoprofen than other NSAIDs. Approximately two-thirds of cases have been associated with fenoprofen. Hence, the structure of the drug itself appears to be of major importance. The syndrome has been attributed, nonetheless, to virtually all NSAIDs, including those from structurally distinct classes.<sup>1,22,39,40,41</sup>

The mechanism of NSAID-induced nephrotic syndrome has not been fully characterized. The association of this syndrome with structurally distinct NSAIDs suggests a common denominator. T lymphocytes may function as immune mediators instead of the humoral factors that are responsible for classic drug-induced allergic interstitial nephritis. In keeping with this hypothesis, NSAID-induced prostaglandin inhibition may play an indirect role. By inhibiting cyclooxygenase, NSAIDs may promote metabolism of arachidonic acid to non-prostaglandin eicosanoids. Indeed, leukotrienes, the products of the interaction between lipoxygenase and arachidonic acid, are known to recruit T lymphocytes and promote the inflammatory process. Leukotrienes may also contribute to proteinuria by increasing vascular permeability.<sup>1,40,41</sup>

### PAPILLARY NECROSIS

Papillary necrosis with interstitial nephritis is a well-known complication of chronic phenacetin abuse that has been reviewed extensively elsewhere.<sup>43</sup> Fortunately, the incidence of the latter complication has diminished considerably because of a better understanding of the pathophysiology and patient education. It has been suggested that chronic aspirin alone may also induce papillary necrosis,<sup>44</sup> but it is not clear that this can actually occur. What is clinically apparent is that chronic (10 to 20 years) exposure of the kidney to high doses of analgesic combinations such as salicylate and acetaminophen (the metabolite of phenacetin), often with the addition of caffeine, can and will produce chronic, progressive papillary necrosis.

The black pigmentation found within necrotic papillae associated with phenacetin abuse (or phenacetin-containing combinations) is absent in patients who ingest aspirin alone or other NSAIDs. This black pigmentation may represent a breakdown product of phenacetin.<sup>43</sup>

In preclinical studies, nearly all of the NSAIDs produced papillary necrosis in experimental animal models. Clinical toxicity is exceedingly rare but has been reported for ibuprofen,<sup>45</sup> phenylbutazone,<sup>46,47</sup>

fenoprofen,<sup>48</sup> and mefenamic acid,<sup>49</sup> and according to prescribing information, several other NSAIDs.

The typical candidate for NSAID-induced papillary necrosis is a middle-aged woman with a history of ingesting over-the-counter, combination analgesics for headache. Closer questioning may reveal that the patient takes the analgesic for the mood-altering effects of caffeine. Renal manifestations may include loin pain, macroscopic hematuria, ureteral obstruction, and/or uremia. Urinary tract infection and hypertension are common secondary findings. Reversibility is determined by the extent of deterioration and ability to discontinue NSAID therapy.<sup>43</sup> Recent reports from the FDA<sup>50</sup> of spontaneous gross hematuria associated with NSAIDs such as ibuprofen (three cases) suggest that papillary necrosis also occurs with newer NSAIDs. These data suggest a minor degree of papillary damage, but chronic progressive deterioration of renal function is not a feature of most reports.

The mechanism of NSAID-induced papillary necrosis is not clear. The causative role of NSAIDs is difficult to delineate because of the presence of confounding factors such as underlying disease, urinary tract infection, and/or concomitant medications. Selected NSAIDs may exert a direct toxic effect on renal papillae, particularly combinations of aspirin and acetaminophen, a major metabolite of phenacetin. Both drugs are highly concentrated in the medulla. Aspirin depletes cellular glutathione, which would otherwise neutralize the acetaminophen metabolite, N-acetyl-benzo-quinoneimine. Without glutathione, this highly reactive metabolite could lead to cell death.<sup>43</sup>

Prostaglandin inhibition may also play a role.<sup>1</sup> Medullary ischemia, a possible precipitating factor in development of papillary necrosis, results from NSAID-induced reduction in blood flow to the renal medulla in experimental models.<sup>51,52</sup>

### OTHER NSAID-INDUCED RENAL SYNDROMES

Phenylbutazone, suprofen, and benoxaprofen produce unique renal syndromes that are of historic interest. These complications are rarely encountered because phenylbutazone use has diminished because of the availability of safer drugs, and suprofen and benoxaprofen have been removed from the market.

Two mechanisms have been identified for phenylbutazone-induced acute oligo-anuric renal failure.<sup>1</sup> Phenylbutazone is known to inhibit uric acid reabsorption, which may cause hyperuricosuria, and ultimately, bilateral ureteral obstruction due to uric acid stones.<sup>53</sup> Secondly, an idiosyncratic reaction has

been reported that results in acute tubular injury without uric acid precipitation.<sup>54</sup> Underlying renal impairment is a risk factor for the latter reaction. Also, patients experiencing this reaction appear to be predisposed to subsequent renal injury from other NSAIDs. These observations suggest that prostaglandin inhibition may play a role in the development of the idiosyncratic reaction.<sup>1</sup>

Suprofen-induced acute renal failure is characterized by acute flank and/or abdominal pain, occurring within 12 hours after starting therapy. In a series of 16 patients described by Hart and colleagues,<sup>55</sup> the mean peak serum creatinine was 3.6 mg/dL (range: 2–8 mg/dL) and was within normal limits at follow-up in most patients. Urinalysis revealed microhematuria (8/12 patients) and proteinuria (7/12 patients) but no crystals. One of our patients with suprofen-induced flank pain syndrome had birefringent crystals in the urine several hours after the injection of the drug and at a time when rehydration had already been commenced. We did not determine if these crystals were uric acid or drug metabolites.

The mechanism of suprofen-induced flank pain and acute renal failure was never conclusively identified before the drug was removed from the market. No obvious risk factors were identified in the previous series since all patients appeared to be in good health and took NSAIDs for acute symptomatic relief. It has been hypothesized that the suprofen flank pain syndrome is related to acute uric acid

crystal precipitation within the nephron leading to acute urinary flow obstruction.<sup>50,55</sup> Suprofen is known to have uricosuric activity. The finding of hyperuricemia (mean: 10.8 mg/dL) in four of four patients suggests that this may be a risk factor.<sup>55</sup>

Benoxaprofen, an NSAID with a long half-life, was removed from the market in 1982, within weeks after its introduction, because of adverse effects. It is remembered for severe hepatic toxicity that occasionally resulted in death; however, renal failure was also a contributing factor. Risk factors for benoxaprofen-induced toxicity were old age and concomitant diuretic therapy, two factors known to increase the risk of acute functional renal failure.

## CONCLUSIONS

NSAIDs are considered safe and suitable for the treatment of a variety of chronic and acute conditions. The risk of renal failure after the initiation of any given NSAID is low; however, the number of at-risk patients is high because of the widespread use of these drugs.

In most cases, NSAID-induced renal syndromes are a direct or indirect result of prostaglandin inhibition, which has important clinical implications. At this time, it is not clear whether it is possible to completely separate the effects of NSAIDs on systemic prostaglandins, which mediate anti-inflammation activity, from renal effects. Thus, under the right cir-

TABLE IV

### Summary of Effects of NSAIDs on Renal Function

Renal Syndrome	Mechanism	Risk Factors	Prevention/Treatment
Sodium retention and edema	↓ Prostaglandin	NSAID therapy (most common adverse effect)	Stop NSAID
Hyperkalemia	↓ Prostaglandin, ↓ potassium to distal tubule and ↓ aldosterone/renin-angiotensin	Renal disease Heart failure Diabetes Multiple myeloma Potassium therapy K <sup>+</sup> -sparing diuretic	Stop NSAID Avoid indomethacin in high-risk patients
Acute deterioration of renal function	↓ Prostaglandin and disruption of hemodynamic balance	Liver disease Renal disease Heart failure Dehydration Old age Fenoprofen	Stop NSAID Avoid use in high-risk patients
Nephrotic syndrome with interstitial nephritis	↑ Lymphocyte recruitment and activation		Stop NSAID Dialysis and (?) steroids as needed
Papillary necrosis	Direct toxicity	Phenacetin abuse Aspirin-acetaminophen combination	Stop NSAID Avoid chronic analgesic use

cumstances, virtually any NSAID can produce renal complications. Fortunately, these complications are usually reversible if the diagnosis is recognized promptly and NSAID therapy is discontinued.

With an understanding of the pathophysiology involved, preventive clinical measures can be put into operation. Risk factors have been identified for most NSAID-induced renal syndromes (Table IV). It is prudent to avoid high-dose, chronic NSAID therapy in at-risk patients (Table III). Unfortunately, this is not always possible. If NSAIDs are necessary in these high-risk groups, the patients should be monitored closely and receive appropriate counselling. Monitoring should begin within a week after initiation of a short-acting NSAID (e.g., ibuprofen) and continue indefinitely for signs of syndromes having delayed onset (e.g., nephrotic syndrome with interstitial nephritis).

In the event of NSAID-induced renal failure, the NSAID should be discontinued promptly. The patient should receive supportive care as needed. After stabilization of renal function, rechallenge with the same dose of the offending drug or even a structurally unrelated NSAID is likely to reproduce the adverse effect. (Patients who have recovered from an episode of protracted dehydration due to diuretics or intercurrent disease are an exception to this rule.) Thus, if anti-inflammatory therapy is mandatory, underlying risk factors should be identified and eliminated, if possible. Unfortunately, this is often not possible, as in the case of old age or chronic heart, kidney, or liver disease. These patients may require alternative therapy using corticosteroids or other supportive drugs such as acetaminophen or colchicine.

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# The Renal Effects of Nonsteroidal Anti-inflammatory Drugs: Summary and Recommendations

William M. Bennett, MD, William L. Henrich, MD, and Jeffrey S. Stoff, MD

● The renal effects of nonsteroidal anti-inflammatory drugs are reviewed with special emphasis on the clinical, pathophysiologic, and risk factors for acute renal failure. Renal papillary necrosis and chronic renal insufficiency can occur with the prolonged use of these drugs, although the prevalence of this manifestation of nonsteroidal anti-inflammatory drug nephrotoxicity is unknown. Current recommendations based on a critical literature survey are provided, along with a list of suggested areas in which more research is needed.  
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**INDEX WORDS:** Nonsteroidal anti-inflammatory drugs; acute renal failure; chronic renal failure; analgesic nephropathy; prostaglandins.

**N**ONSTEROIDAL anti-inflammatory drugs (NSAIDs) are popular and used widely because of their acknowledged efficacy and excellent safety profile in a wide range of clinical conditions. Despite their many useful therapeutic applications, there is now substantial evidence arising from experimental studies and clinical studies in humans for multiple effects of NSAIDs on kidney function. This is not surprising since the principal action of NSAIDs is to block the synthesis of cyclo-oxygenase products of arachidonic acid, which have a critical modulatory role on renal hemodynamics, renal epithelial cell fluid and ion transport, and the synthesis and action of renal hormones. Nonsteroidal anti-inflammatory drugs are now available both in over-the-counter and prescription strengths. The majority of healthy, normal subjects who ingest therapeutic dosages of NSAIDs for limited duration tolerate these drugs without adverse effects. However, a subset of individuals are susceptible to subclinical as well as serious renal toxicity from these agents. In addition to the effects listed in Table 1, NSAIDs interfere with the efficacy of antihypertensive medicines, leading to an increase in blood pressure.

Since the toxicity of NSAIDs in the kidney is linked to the disruption of renal prostaglandin

synthesis, a brief review of the renal effects of prostaglandins and the consequences of synthesis interruption is in order.

## PROSTAGLANDIN SYNTHESIS AND COMPARTMENTALIZATION

Prostaglandins are derivatives of arachidonic acid, a 20-carbon tetraenoic acid, which is acylated to membrane phospholipids. Deacylation of arachidonic acid from the cell membrane is controlled by phospholipases, predominantly phospholipase A<sub>2</sub>. Vasopressin,<sup>1</sup> bradykinin,<sup>2</sup> angiotensin,<sup>3</sup> and norepinephrine<sup>4</sup> all stimulate arachidonic acid release from membranes, whereas glucocorticoids inhibit release.<sup>5</sup> After arachidonic acid is released from the cell membrane, several synthetic pathways are then available. Molecular oxygen may be added to the arachidonic acid by the action of an intracellular endoplasmic reticulum-bound peroxidase enzyme (cyclo-oxygenase), which leads to the synthesis of endoperoxide PGG<sub>2</sub>. A second endoperoxide (PGH<sub>2</sub>) is then formed with the liberation of a superoxide radical. Once formed, PGH<sub>2</sub> has a short half-life and is rapidly acted on by a series of enzymes that produce the biologically active molecules. Nonsteroidal anti-inflammatory drugs exert their prostaglandin inhibitory effects by primarily inhibiting the activity of cyclo-oxygenase by 70% to 95%. Prostaglandin biosynthesis is also decreased by NSAIDs, reducing the generation of superoxide and hydroxyl-free radicals.<sup>6,7</sup>

The endoperoxide PGH<sub>2</sub> is transformed by a series of enzymes to the dienoic series of prostaglandins. These prostaglandin metabolites possess biologic activity in the kidney; for example, prostacyclin synthetase acts to form prostacyclin (PGI<sub>2</sub>), whereas thromboxane synthetase forms thromboxane (TXA<sub>2</sub>) and the isomerases act to

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Table 1. Kidney Manifestations of NSAIDs

Kidney Toxicity	Mechanism	Risk Factors
Acute renal failure	Loss of counterregulatory prostaglandins	Plasma volume contraction, congestive heart failure, cirrhosis, and ascites
Sodium retention	Loss of natriuretic prostaglandins	Unknown
Potassium retention	Hyporeninemic hypoaldosteronism	Concomitant defects in potassium homeostasis
Water retention	Enhanced antidiuretic hormone action, increased medullary tonicity	Unknown
Acute interstitial nephritis	Reactive arachidonic acid metabolite	Unknown

form  $\text{PGE}_2$  and  $\text{PGF}_2$ . Prostaglandins are known to exert physiologic effects at the locations at which they are synthesized. In this regard, they are really autoids rather than true hormones. Prostaglandins that are excreted into renal lymph or into the renal vein are rapidly metabolized into active products in the lung. The prostaglandin synthetic pathway is shown in Fig 1. Prostaglandins synthesized in the renal cortex regulate renal cortical processes (renal vascular resistance and renal secretion), whereas prostaglandins formed in the medulla modulate medullary physiologic events (salt and water handling). The most abundant prostaglandin found in the tubules is  $\text{PGE}_2$ . The cortical and particularly medullary portion of the collecting duct are the dominant sites of  $\text{PGE}_2$  synthesis. Medullary interstitial cells are also a rich source of  $\text{PGE}_2$  production. Prostaglandin  $\text{E}_2$  undergoes spontaneous hydrolysis to 6-keto- $\text{PGF}_{1\alpha}$ . Prostaglandins are rapidly metab-

olized into inactive products by a 15-prostaglandin dehydrogenase.

#### EFFECTS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON RENAL FUNCTION: CLINICAL CONSEQUENCES

Under baseline and euvoletic circumstances there is typically a very low rate of prostaglandin synthesis. Because this is true in a healthy state, it is difficult to demonstrate that prostaglandins contribute to the normal maintenance of renal function even when using powerful cyclo-oxygenase inhibitors, such as NSAIDs. When prostaglandin synthesis is upregulated as hemodynamic destabilization occurs, the synthesis and release of prostaglandins is greatly enhanced. Under these circumstances the inhibition of prostaglandin synthesis has been clearly demonstrated to have profound adverse hemodynamic effects on the kidney. Most of these effects have been seen

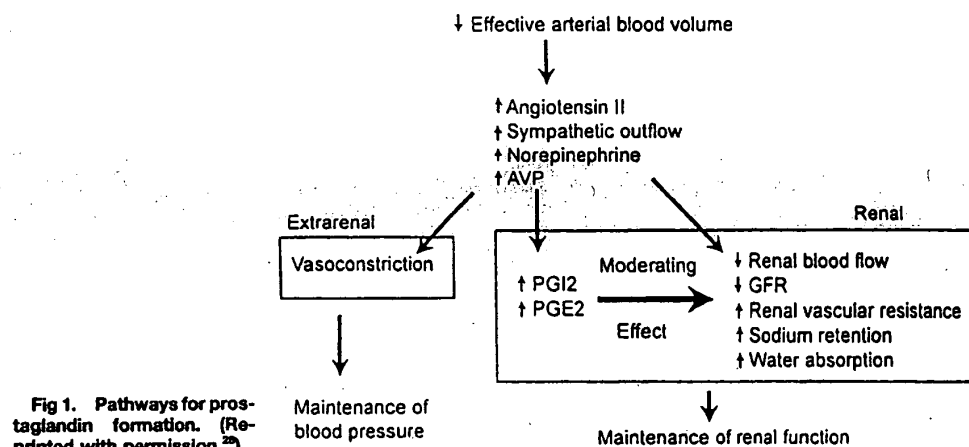


Fig 1. Pathways for prostaglandin formation. (Reprinted with permission.<sup>20</sup>)

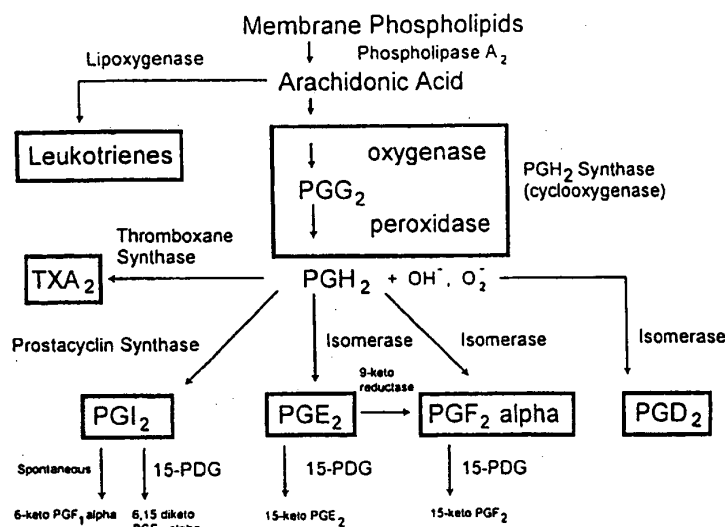


Fig 2. Schematic depiction of the relationship between vasodilator and vasoconstrictor input into the kidney. PGI<sub>2</sub> and PGE<sub>2</sub> exert a moderating effect on renal vasoconstrictive stimuli.

in circumstances in which blood volume or effective arterial blood volume is compromised and vasoconstrictor peptide secretion would be expected to be high. Angiotensin II, norepinephrine, vasopressin, and sympathetic nerve activity all increase under these perturbed circumstances and cause an increase in renal vascular resistance. In addition, each of these stimuli is a potent agonist for prostaglandin synthesis.<sup>2,3,8</sup> Hence, what ensues is a dynamic interplay between counterbalancing vasoconstrictor and vasodilator forces. It is under these circumstances that the inhibitor of prostaglandin synthesis will result in excessive vasoconstriction, with a consequent decrease in renal blood flow and finally a decrement in glomerular filtration rate. These relationships are graphically depicted in Fig 2.

#### ACUTE RENAL FAILURE

Acute renal failure (ARF) due to a decrease in renal blood flow secondary to increase renal vascular resistance has been well described. The afferent renal arteriole appears to be under tonic regulation by vasodilator prostaglandins, and loss of these dilators leads to vasoconstriction and a decrease in glomerular capillary pressure, resulting in a prompt decline in glomerular filtration rate. This form of renal failure is often sudden, presenting with oliguria and a decrease in

fractional sodium excretion. Withdrawal of NSAIDs usually leads to prompt reversal of the ARF. Virtually all NSAIDs have been implicated, although some subclasses of NSAIDs may be less toxic because of renal conversion of active drug to inactive metabolite.

A common risk factor of ARF is the physiologic state of plasma volume depletion induced either by hemorrhage, salt loss, or hypoalbuminemia. In these conditions, circulating vasoconstrictors are released, maintaining vascular resistance and blood pressure at the potential expense of regional organ blood flow. To maintain blood flow, particularly in the kidney, counterregulatory renal prostaglandins are released that counteract vasoconstrictors and normalize renal blood flow. Nonsteroidal anti-inflammatory drugs taken under these circumstances blunt this counterregulatory response and intensify the renal vasoconstriction leading to ARF. If the vasoconstriction is sufficiently intense and of extended duration, acute tubular necrosis may ensue. Similar physiology to intravascular volume depletion is seen in severe congestive heart failure (New York Heart Association grade III, IV) and hepatic failure with ascites. In these two pathophysiologic states, which are also associated with activation of circulating neurohumoral vasoconstrictors, NSAID use may lead to ARF by augmenting arteriolar constriction.

#### INTERRUPTION OF RENAL TUBULAR ION AND WATER TRANSPORT: CLINICAL CONSEQUENCES

Eicosanoids or oxygenated metabolites of arachidonic acid exert modulatory influences on many ion transport sites along the nephron. Consequently, their synthesis interrupted by NSAID use leads to a wide variety of disorders of ion transport. Most prominent among these in clinical use is the retention of sodium. Virtually all individuals will develop positive sodium retention following the use of NSAIDs and escape from this antinatriuretic effect in several days. A small subset of individuals fail to escape and develop a severe edema state. Natriuresis rapidly ensues once the drug is discontinued.

An issue related to sodium retention is the effect of NSAIDs to antagonize the effect of concomitant diuretic use. This antagonism has been described for the use of both thiazide and loop diuretics. Potassium-sparing diuretics, particularly triamterene, have been implicated as a potential risk factor for NSAID-induced ARF. Reports of this combination of drugs have been in the form of case reports and require further study to document the precise risk.

Hyperkalemia is the second major electrolyte disorder that accompanies NSAID use. Since plasma potassium is tightly regulated by several different effector systems, NSAID-induced hyperkalemia seldom occurs in the absence of other defects in potassium homeostasis. The mechanism of NSAID action is the suppression of prostaglandin-mediated renin release leading to a state of hyporeninemic hypoaldosteronism. Patients at risk are those on drugs that block internal potassium homeostasis (beta blockers, alpha agonists) or drugs that reduce potassium excretion (potassium-sparing diuretics, aldosterone antagonists). Insulin-dependent diabetic patients, especially with renal dysfunction, as well as patients with moderate to severe renal failure (glomerular filtration rate < 30 mL/min) are at particularly high risk.

Hyponatremia secondary to a defect in free water clearance is well documented in the use of NSAIDs. Abundant evidence indicates that prostaglandins antagonize the hydro-osmotic effect of antidiuretic hormone. Thus, NSAID use enhances antidiuretic hormone action and promotes water retention. This effect is further accentuated

by the effect of NSAIDs to augment medullary tonicity by enhancing the active transport of chloride at the thick ascending limb of the loop of Henle. Restriction of water intake may be necessary in those patients who develop hyponatremia during NSAID use.

#### ACUTE INTERSTITIAL NEPHRITIS AND MINIMAL-CHANGE GLOMERULOPATHY

Nonsteroidal anti-inflammatory drugs of all classes have been reported to induce a syndrome of acute interstitial nephritis with or without minimal-change glomerulopathy. This rare syndrome has been reported after 2 to 18 months of NSAID therapy and may be sufficiently severe as to require dialysis support. Most cases are reversible and are characterized pathologically by a mononuclear cell infiltrate of lymphocytes and plasma cells. When there is glomerular involvement, the predominant lesion is epithelial cell podocyte fusion detected by electron microscopy. The most culpable NSAID appears to be fenoprofen, although virtually all NSAIDs have been reported to induce this pathology. Acute interstitial nephritis is probably the most common presentation, followed by combined interstitial and glomerular disease; the least common is minimal-change glomerulopathy. The usual stigmata of an allergic syndrome are absent, such as skin rash, peripheral eosinophilia, and increased immunoglobulin E level, suggesting that the mechanism of action may be related to a reactive non-cyclo-oxygenase product of arachidonic acid metabolism. The syndrome is usually reversible by the withdrawal of the offending NSAID. There are no controlled studies supporting the use of corticosteroids to alter the rate or extent of renal recovery.

#### NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND CHRONIC RENAL DISEASE

Despite the well-characterized acute biologic effects of NSAIDs on the kidney, there are no scientifically acceptable data documenting the safety of this class of drugs on renal structure and function when taken chronically. Epidemiologic data show an 8.8 increased relative risk of end-stage renal disease in subjects ingesting 5,000 or more doses of NSAIDs compared with control subjects matched for age. However, these data are flawed by the study design and do not neces-

sarily support a cause and effect relationship.<sup>9</sup> In a better-designed, multicenter, case control study, the risk of chronic renal disease defined as a serum creatinine of  $\geq 1.5$  mg/dL was 2.1 (95% confidence interval, 1.1 to 4.1) in daily users of NSAIDs.<sup>10</sup>

The hallmark lesion of analgesic-associated nephropathy is renal papillary necrosis, which can lead to progressive renal failure but also may be present with a well-preserved glomerular filtration rate, making ascertainment of cases by renal function studies alone problematic. In a prospective radiographic study of 259 patients with an intake of 1,000 to 26,000 NSAID doses, papillary necrosis was found in 38 users who took predominately physician-prescribed NSAIDs. Only 65% of these patients had renal functional impairment. Thus, it is clear that long-term use of NSAIDs can cause renal papillary necrosis and renal insufficiency.<sup>11</sup> The frequency of renal papillary necrosis as a primary or contributing cause of end-stage renal disease is unknown because of the infrequent radiographic diagnosis by physicians resulting in misclassification and the insensitivity of renal diagnosis by currently available renal function tests, such as serum creatinine. Furthermore, other known effects of NSAIDs on the kidney, including increased blood pressure and renal hemodynamic changes, could contribute to facilitating progressive renal disease of other etiologies. The experimental production of renal papillary necrosis by NSAIDs is enhanced by caffeine.<sup>12</sup> It is not known whether this is clinically relevant because caffeine intake has not been considered in epidemiologic or other clinical studies.

While there is an extensive package insert documenting the renal consequences of prescription NSAIDs, there are no renal warnings at all on over-the-counter NSAIDs, which are heavily advertised to the public. Thus, patients in high-risk groups or patients with pre-existing kidney disease could be unaware that they have been exposed to these drugs. Case reports and case series document the ability of a variety of chemically unrelated NSAIDs to produce renal papillary necrosis and renal insufficiency.<sup>13-16</sup>

There are other causes of chronic renal failure in patients using prescription or over-the-counter NSAIDs. Although acute renal dysfunction due to NSAIDs is most often reversible, approximately 20% of reported cases have permanent

renal failure whether the NSAIDs produced ARF via acute tubular necrosis, acute interstitial nephritis with proteinuria, or simply renal blood flow decreases in high-risk populations.<sup>17</sup>

Irreversible renal failure may also occur in children.<sup>18</sup> Prenatal exposure to indomethacin may lead to severe irreversible renal failure, which is favored by prior stimulation of the renin-angiotensin system. Since these infants are not generally candidates for renal replacement, the consequences of NSAIDs in this setting are not reflected in the end-stage renal disease statistics of the US Renal Data System. In recent series, neonatal renal failure deaths were reported with 150 to 400 mg of indomethacin per day for 2 to 11 weeks during pregnancy.<sup>19-22</sup> Low birth weights and hyperkalemia also have been described in surviving infants.<sup>23</sup>

When NSAIDs are used to reduce proteinuria in nephrosis, permanent renal damage has been reported. Another potential adverse effect of NSAIDs in patients with chronic renal failure includes fatal hyperkalemia from drug-drug interactions with angiotensin-converting enzyme inhibitors, potassium-sparing diuretics, or beta blockers.<sup>24</sup>

Although the population exposure to prescription and nonprescription NSAIDs is large, even the estimated 1% patients with clinically detectable renal dysfunction has important medical and economic implications.<sup>25</sup> The longest period of observation with regard to chronic NSAID usage is 6 to 12 months. In the United States, there are no cross-sectional or prospective studies applied to NSAIDs using the objective criteria for analgesic nephropathy diagnosis proposed by Elseviers and DeBroe,<sup>26</sup> although these criteria have been validated in Europe. In a large general internal medicine practice in which records of analgesic users were surveyed, patients older than 65 years and those with coronary artery disease were at risk of renal impairment with NSAIDs compared with users of acetaminophen. No radiographic data are available.<sup>27</sup>

#### SUMMARY

Nonsteroidal anti-inflammatory use in the general population is safe and efficacious when used in therapeutic dosages for a limited period of time. In contrast, patients with pre-existing risk factors are susceptible to potentially life-threat-

ening toxicities, including ARF and serious fluid and electrolyte disorders. Numerous studies have delineated the mechanism(s) by which NSAIDs induce these adverse effects and identify the patients at highest risk (Table 1). The safe use of these agents requires the identification of these risk factors, interventions to ameliorate these risks when possible, and the careful monitoring of renal function and electrolyte concentrations to avoid serious complications.

Renal papillary necrosis and chronic renal insufficiency can occur secondary to prolonged use of prescription and over-the-counter NSAIDs. Neonatal renal failure and renal death may occur from use during pregnancy. While acute renal failure due to NSAIDs occurs in well-defined high-risk patients or under rare idiosyncratic circumstances, renal recovery is incomplete in approximately 20% of reported cases. There are epidemiologic data to support NSAID use as a risk factor for chronic renal dysfunction, even end-stage renal disease, in a cumulative dose-dependent fashion. Despite over-the-counter status, there are no long-term studies of renal structure or function that document the safety of these drugs.

### CONCLUSIONS

1. Use of NSAIDs in the general population is safe and effective when used in therapeutic dosages for a limited period of time.
2. Patients with pre-existing risk factors are susceptible to potentially life-threatening toxicities, including ARF and serious fluid and electrolyte disorders.
3. Renal papillary necrosis and chronic renal failure can occur secondary to prolonged use of prescription and over-the-counter NSAIDs.
4. Neonatal renal failure and renal death may occur from NSAID use during pregnancy.
5. Nonsteroidal anti-inflammatory drug-induced ARF is usually, but not inevitably, reversible.
6. There are no acceptable epidemiologic or clinical data regarding the risk of NSAIDs for chronic renal failure, renal papillary necrosis, or end-stage renal disease.
7. There are no data of NSAIDs' effect on progression of other renal diseases (experimental or clinical).

### RECOMMENDATIONS

1. There should be an explicit label to warn patients taking over-the-counter NSAIDs of potential renal toxicities (similar to that suggested in *Am J Kidney Dis* 6:4-5, 1985).
2. Design and implement properly controlled studies on the renal and cardiovascular safety of chronic NSAIDs by themselves or in the presence of other known etiologies of renal disease.
3. Combinations of NSAIDs with other analgesics and/or caffeine should be prospectively evaluated for renal safety prior to release.

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## Effects of NSAIDs on the kidney.

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NSAID use is pervasive in our society. Existing NSAIDs pose little risk to patients who tolerate them early during their administration. Among persons with normal renal function who have no other risk factors (dehydration) for an acute hemodynamic effect, there is no risk. However, NSAID administration to susceptible persons may cause decrements in renal plasma flow and glomerular filtration rate within hours. This acute hemodynamic effect is the most common renal syndrome caused by NSAIDs. With careful monitoring, this effect is readily detected with routine clinical laboratory (serum creatinine and/or blood urea nitrogen concentrations). However, patients who continue administration of NSAIDs in this setting risk acute tubular necrosis and permanent damage to the kidney. Newer NSAIDs that selectively inhibit cyclooxygenase-2: cyclooxygenase-1 ratio may provide more favorable risk profile for patients who cannot tolerate existing drugs.

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